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SHORT COMMUNICATION

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Polymorphisms of androgens-related genes and idiopathic male infertility in Turkish men

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Abstract

Androgens, testosterone and dihydrotestosterone (DHT) are endocrine regulators of spermatogenesis and act via androgen receptor (AR). The aim of this study was to investigate the association(s) of *AR* (CAG repeat length), *SRD5A2* (rs523349, V89L) and *TNF-α* (rs1800629, -308G/A) polymorphisms with idiopathic male infertility in Turkish men. This case-control study consisted of 312 men with idiopathic infertility and 113 fertile men. Polyacrylamide gel electrophoresis (PAGE) or PCR-restriction fragment length polymorphism methods were used for genotyping. The mean *AR* CAG repeat length was significantly longer in infertile men than in fertile men ($p = 0.015$). However, there was no significant association between the *SRD5A2* genotypes (VV, VL and LL) and the risk of infertility ($p = 0.516$). The genotype frequency and allele distribution of *TNF-α* -308G/A polymorphism (GG, GA, AA genotypes and G, A alleles) were not associated with male infertility ($p = 0.779$ and $p = 0.743$ respectively). *AR* CAG repeat expansion might be one of the risk factors for idiopathic male infertility in Turkish men. Further studies investigating the association of male infertility with *AR* CAG, V89L and -308G/A polymorphisms are warranted to understand the possible associations among them.

KEYWORDS

AR CAG repeat length, male infertility, *SRD5A*, *TNF-α*

1 | INTRODUCTION

Spermatogenesis is an extraordinary regulated process via endocrine, physiological and molecular pathways (Krausz & Sassone-Corsi, 2005). Androgens involved in the endocrine regulation of spermatogenesis are essential for sexual differentiation and male gametogenesis (Holdcraft & Braun, 2004) with the biological function of androgens (e.g. testosterone) being dependent on the androgen receptor (AR) encoded by the *AR* gene (Akinloye et al., 2009). The *AR* gene comprises polymorphic CAG trinucleotide repeats coding polyglutamine stretch in the first exon of the gene (Bogaert et al., 2009). CAG repeat polymorphism has been reported to be associated with prostate cancer and male infertility (Delli Muti et al., 2014; Yoo et al., 2014).

Dihydrotestosterone (DHT) is one of the bioactive androgens involved in normal spermatogenesis and male reproductive function (Serdarogullari et al., 2021; Ye et al., 2011; Zhao et al., 2012). DHT derives from testosterone by the action of 5α-reductase isoenzymes and binds to the same AR as testosterone, but with a higher affinity. Two types of 5α-reductase isoenzymes, type-1 and type-2, present in human beings are coded by *SRD5A1* and *SRD5A2* genes respectively (Zhao et al., 2012). Several *SRD5A2* gene polymorphisms that cause amino acid substitutions and alterations in enzyme activity have been identified including C5R, P48R, A49T, A51T, V89L, T187M and F234L (Makridakis et al., 2000). *SRD5A2* V89L polymorphism has been suggested to cause decrease in the enzyme activity of 5α-reductase (Makridakis et al., 1997), culminating the abnormal spermatogenesis (Zhao et al., 2012).

Germ cell development is initiated and supported by Sertoli cells after testosterone stimulation of the AR and modulation of the AR expression has been proposed as a vital regulator mechanism of Sertoli cell responsiveness to testosterone (Oduwole et al., 2018). Nuclear factor kappa B (NFκB) and NFκB-activating cytokine TNF-α have been reported to stimulate AR expression as crucial factors for the maintenance of mammalian spermatogenesis (Delfino et al., 2003). TNF-α is a well-known cytokine involved in the spermatogenesis (Bami et al., 2017). Infertile men with asthenozoospermia have higher expression of TNF-α than fertile men (Ghandehari-Alavijeh et al., 2019). On the other hand, a dose- and time-dependent adverse effect of TNF-α on sperm function and quality as well as DNA and chromatin integrity has been reported (Pascarelli et al., 2017; Said et al., 2005). The -308 G/A polymorphism in the promoter region of *TNF-α* gave rise to increase in the expression of TNF-α and has been associated with poor semen quality (Tronchon et al., 2008).

Smoking is known to be a risk factor for sperm quality and fertilisation ability (Gunes et al., 2018). Smoking has also been suggested to cause an increase in the plasma level of TNF-α (Tappia et al., 1995) and to associate with number of CAG repeats in the *AR* gene (Mitra et al., 2012). Infertile male smokers had low level of SRD5A2 enzyme in their serum (Mohammad & AL-Azzawie, 2020).

SRD5A2 and *TNF-α* polymorphisms have been associated with AR and/or androgens as well as spermatogenesis (Bami et al., 2017; Moura-Massari et al., 2016; Shukla et al., 2013; Zalata et al., 2013; Zhao et al., 2012). TNF-α stimulates AR expression, AR-CAG repeat expansion affects the transcription activity of *AR* (Tut et al., 1997), and *SRD5A2* V89L polymorphism decreases the enzyme activity of SRD5A2, which affects the conversion of DHT binding same AR with testosterone (Makridakis et al., 1997). Therefore, polymorphisms of *AR*, *SRD5A2* and *TNF-α* may affect male infertility risk. To the best of our knowledge, no study investigated the association of *TNF-α* -308G/A polymorphisms with idiopathic infertility in Turkish men. Therefore, this study aimed to investigate whether there is an association between CAG repeat expansion, *SRD5A2* V89L and *TNF-α* -308G/A polymorphisms and idiopathic infertility in Turkish men. Moreover, the effect of smoking status on polymorphisms of *AR* and *TNF-α* as well as correlation(s) between polymorphisms of *AR*, *SRD5A2* and *TNF-α* genes, levels of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were also evaluated in the study population.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was ethically approved by the Institutional Review Board of Ondokuz Mayis University (OMU) (ID Number: KAEK 2016/215). All participants signed an informed consent form to participate in this study. All subjects who enrolled in the study had same ethnic origin and were from the Central Black Sea Region in Turkey.

In this study, 312 idiopathic infertile men [33.19 ± 6.6, (mean age ± *SD*)] with sperm counts $<5 \times 10^6$/ml attending the Andrology Clinic of OMU were recruited. One hundred sixty-nine infertile patients were diagnosed with non-obstructive azoospermia (NOA) and 69 oligoasthenoteratozoospermia (OAT). Semen analysis results were not available in 75 infertile men with sperm counts less than 5×10^6/ml. To determine the infertility status, at least three semen analysis were performed after 2–5 days of sexual abstinence time. Infertile men with numerical and structural chromosomal aberrations, Y-chromosome microdeletions, *CFTR* mutations, testicular malignancies, trauma, infection or surgery and any other known causes of male infertility were excluded from the study. Controls (n = 113) were fertile men [36.65 ± 8.6, (mean age ± *SD*)] who had at least one child without benefiting from assisted reproductive technologies. Conception occurred in the female partner of all controls within 12 months of trying. Demographic data and smoking status of the participant were obtained by a questionnaire. Study participants were divided into two groups as regards smoking status, ever (n = 86) and never smokers (n = 162). Ever smokers are men who smoked more than 100 cigarettes during their lifetime while never smokers smoked less than 100 cigarettes during their lifetime. In infertile group, 72 men were ever smokers and 105 men were never smokers; in the fertile group, those were 14 and 57 respectively. Data regarding testicular volume and FSH, LH and total testosterone levels were retrospectively acquired.

2.2 | Semen analysis

Semen samples were obtained from participants by masturbation after 2–5 days of sexual abstinence time and analysed according to the latest guideline of the World Health Organization (WHO, 2010). After complete liquefaction of semen samples at 37°C for 20–30 min, sperm count, concentration and motility were evaluated using a Microcell counting chamber (Vitrolife). Sperm morphology was evaluated according to strict criteria (Kruger) after diff-quick staining.

2.3 | Genomic DNA isolation

Genomic DNA was isolated from peripheral venous blood samples of the participants by using a salting out method (Miller et al., 1988).

2.4 | *AR* CAG repeats length analysis

The CAG repeat region of the *AR* gene was amplified by polymerase chain reaction (PCR) of genomic DNA using primers as described previously (Tanaka et al., 1996). In brief, CAG repeat amplification was performed in a 25 μl reaction volume

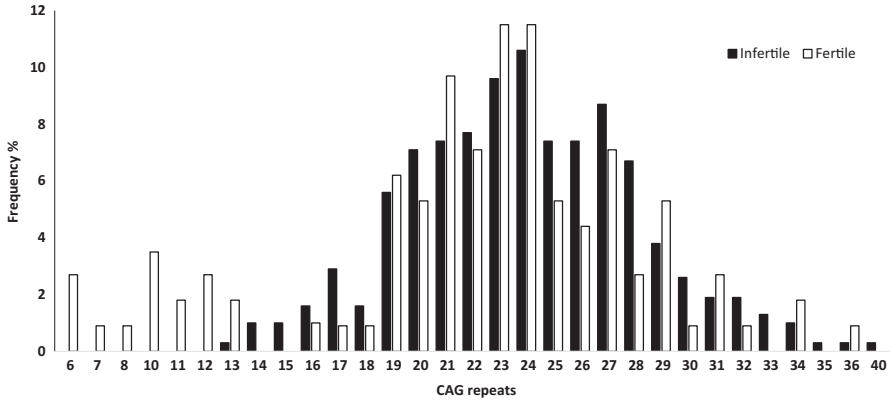

FIGURE 1 Distribution pattern of *AR* CAG repeats in idiopathic infertile men ($n = 312$) and fertile controls ($n = 113$). There were 31 CAG alleles, ranging from 6 to 40 repeats in the total study population, which was 25 CAG alleles (13–40 CAG repeats) in infertile group and 26 CAG alleles (6 to 36 CAG repeats) in fertile group

including ~100 ng genomic DNA, 0.4 μM sense and antisense primers (*AR*-F: 5′-TGCGCGAAGTGATCCAGAACC-3′ and *AR*-R: 5′-CTCATCCAGGACCAGGTAGCC-3′), 1X PCR buffer [200 mM Tris HCl (pH 8.4), 500 mM KCl], 1.5 mM $MgCl_2$, 200 μM dNTP, 5% DMSO and 2 U *Taq* DNA polymerase (Thermo Fisher Scientific). PCR amplification was carried out according to Tanaka et al. (1996) with some modifications. After the initial denaturation at 98°C for 5 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 30 s and elongation at 72°C for 45 s followed by a final extension at 72°C for 10 min were performed (Tanaka et al., 1996). The PCR amplicons were evaluated on 8% polyacrylamide gel electrophoresis (PAGE) stained with ethidium bromide. The length of CAG repeat was measured by using 20 bp ladder with Vision Capture Program (Wilber Lourmat, Marne-la-Vallee, France).

2.5 | Genotyping of *SRD5A2*

SRD5A2 gene V89L polymorphism was assessed using PCR and restriction fragment length polymorphism (RFLP) with certain modifications (Yamada et al., 2001). Briefly, V89L polymorphic region of *SRD5A2* gene was amplified in a final volume of 25 μl containing ~100–150 ng genomic DNA, 5 μM forward and revers primers (*SRD5A2*-F: 5′-GCAGCGGCCACCGGCGAGG-3′ and *SRD5A2*-R: 5′-AGCAGGGCAGTGCGCTGCACT-3′), 1X PCR buffer [200 mM Tris HCl (pH 8.4), 500 mM KCl], 1.5 mM $MgCl_2$, and 200 μM dNTP, 8% DMSO and 2 U Taq DNA polymerase (Thermo Fisher Scientific). V89L polymorphism was detected by PCR cycling after the initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and elongation at 72°C for 1 min followed by a final extension at 72°C for 10 min. Digestion of PCR amplicons was carried out using 10 U RsaI enzyme (Thermo Fisher Scientific) overnight at 37°C. *SRD5A2* genotypes were distinguished on a 3% Nu Microphor agarose gel (Prona) by four distinct banding patterns (169, 105, 83 and 64 bp): VV (169, 105, 64 and 19 bp), VL (169, 105, 83, 64 and 19 bp) LL (169, 105 and 83 bp) and genotypes depending on the presence or absence of the RsaI restriction site.

2.6 | Genotyping of *TNF-α*

To evaluate *TNF-α* -308G/A polymorphism, a modified PCR-RFLP method was applied (Jiffri & Elhawary, 2011). Sequences of the primers used for the *TNF-α* gene amplification were *TNF-α*-F: 5′-GAGGCAATAGGTTTTGAGGGCCAT-3′ and TNF-α-R: 5′-GGGACACACAAGCATCAAG-3′ and the restriction enzyme was NcoI (Bioron, Römerberg) used for the RFLP analysis. The cycling conditions of the PCR programme were as follows: initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 20 s, annealing at 58°C for 20 s and elongation at 72°C for 40 s followed by a final extension at 72°C for 10 min. Subsequently, the amplicons were digested overnight at 37°C with 10 U NcoI restriction enzyme. The RFLP products were then separated on a 3% agarose gel (147, 126 and 21 bp) and stained with ethidium bromide. The absence of the 126 bp and 21 bp bands and presence of 147 bp bands was noted as AA genotype.

2.7 | Statistical analysis

Data were statistically analysed using MedCalc Statistical Software version 19.2 (MedCalc Software Ltd). To determine normal distribution of the data, the Kolmogorov-Smirnov test was used. Employment of subsequent tests, parametric or nonparametric, depended on the normal distribution of the data. Genotype and allele frequencies of *SRD5A2* and *TNF-α* genes were evaluated by using Chi-Square test. The associations of V89L and TNF-α polymorphisms and smoking status, age and testis volume were also analysed by Chi-Square test. The association between CAG repeat number and infertility was analysed by means of the Mann–Whitney *U* test. For analysis of all correlations, a Spearman rank correlation was performed. Confidence intervals (CI) value of odds ratio (OR) was 95% and statistically significant *p*-value was lower than 0.05.

3 | RESULTS

In this study, we investigated the association(s) between polymorphisms of *AR*, *SRD5A2* and *TNF-α* genes with idiopathic male infertility. *AR* CAG repeat length was found to be significantly longer

in infertile men than in fertile men [(mean ± *SD*) 24.03 ± 4.33 and 22.05 ± 6.22, respectively, $p = 0.015$]. The most common CAG allele length was 24 CAG repeats in 33 infertile men (10.6%), while the most common alleles were 23 and 24 CAG repeats in fertile men presented in 13 men (11.5%; Figure 1). CAG repeat length demonstrated a similar distribution pattern among infertile men with NOA and OAT (23.36 ± 4.24 and 24.41 ± 4.15, respectively, $p = 0.180$). In the OAT group, the amplification analysis of the *AR* CAG polymorphism revealed 18 alleles with 17–35 repeats, while 22 alleles with 13–40 repeats were observed in the NOA group. The predominant alleles were the same (24 CAG repeats) in both infertile men with NOA ($n = 18$, 3.9%) and OAT ($n = 24$; 10%; Figure 2).

Based on the mean value of the fertile controls, we categorised the CAG repeats as short ≤22 or long repeats >22 (Mifsud et al., 2001). Infertile men did not show any significant frequency differences compared to the controls between short CAG repeats (36.2% vs. 45.1%; $p = 0.2808$) and long CAG repeats (63.8% vs. 54.9%; $p = 0.2090$). Frequencies of short and long CAG repeats were also not different in infertile men with NOA and OAT ($p = 0.6265$ and $p = 0.7593$ respectively). The frequency of short CAG repeats was 39.9% in men with NOA and 34.8% in men with OAT, whereas the long CAG repeat frequency was found in 60.1% nonobstructive azoospermic men and in 65.2% oligoasthenoteratozoospermic men.

No significant ($p = 0.2633$, $p = 0.2911$, $p = 0.5225$, $p = 0.5883$ respectively) difference was observed between the infertile and control groups regarding the correlation of AR CAG repeat polymorphism with total testosterone ($p = 0.4474$ and $p = 0.2936$ respectively), LH ($p = 0.6739$ and $p = 0.3319$ respectively) and FSH levels ($p = 0.1314$ and $p = 0.9937$ respectively) as well as the smoking status ($p = 0.9287$ and $p = 0.4862$ respectively).

Genotype and allele frequencies of *SDR5A2* V89L and *TNF-α* -308G/A polymorphisms in infertile men and controls are presented in Table 1. Genotype frequencies of V89L and -308G/A polymorphisms were in Hardy-Weinberg equilibrium (HWE) in fertile controls ($\chi^2 = 0.763$, $p = 0.683$ and $\chi^2 = 0.082$, $p = 0.960$ respectively). No significant difference was observed in the distribution of V89L polymorphism regarding genotypes and alleles (VV, VL, LL genotypes and V, L alleles) between infertile and fertile men ($p = 0.516$ and $p = 0.460$ respectively). The association analysis of V89L polymorphism with the smoking status ($p = 0.960$) and testis volume did not reveal a statistically significant difference between the two groups ($p > 0.05$). The genotype frequency and allele distribution of *TNF-α* -308G/A polymorphism (GG, GA, AA genotypes and G, A alleles) were found not to be associated with male infertility ($p = 0.779$ and $p = 0.743$ respectively).

Genotype and allele frequencies of the V89L and -308G/A polymorphisms in infertile men with NOA and OAT are given in Table 2. No significant association was observed between the genotype distribution of V89L and TNF-α-308G/A in NOA and OAT genotypes. No significant correlation(s) were observed among the endocrine hormone levels (testosterone, LH and FSH), smoking status and SRD5A2 V89L polymorphism.

We used a Spearman rank (rho) test for correlation between CAG repeat length, V89L and TNF-α -308G/A polymorphisms as doubly; however, we did not find any association among these polymorphisms in infertile men (CAG repeat length and TNF-α -308G/A polymorphisms, $r = 0.0827$, $p = 0.1451$; CAG repeat length and V89L, $r = -0.0804$, $p = 0.1567$; V89L and TNF-α -308G/A polymorphisms, $r = -0.0155$, $p = 0.7854$).

4 | DISCUSSION

In this study, we found a significant association between the *AR* CAG repeat length polymorphism and idiopathic infertility in Turkish men. This finding is supported by studies reporting a significant association between male infertility and CAG repeat expansion (Ashraf et al., 2020; Borjian Boroujeni et al., 2018; Giagulli et al., 2014; Mobasseri et al., 2018). We observed that idiopathic infertile men displayed longer CAG repeats than the fertile controls. Longer CAG repeats are known to cause downward alterations in the transcriptional activity of the AR in vitro (Tut et al., 1997) and have been reported to be associated with an increased risk of male infertility (Mifsud et al., 2001). Androgen and AR have roles in the stimulation or inhibition of spermatogenesis-related genes' expression. The structure and the function of AR molecule and its cofactors depend on the number of glutamine amino acids (CAG repeat number; Pan et al., 2016). AR CAG repeat polymorphism may affect

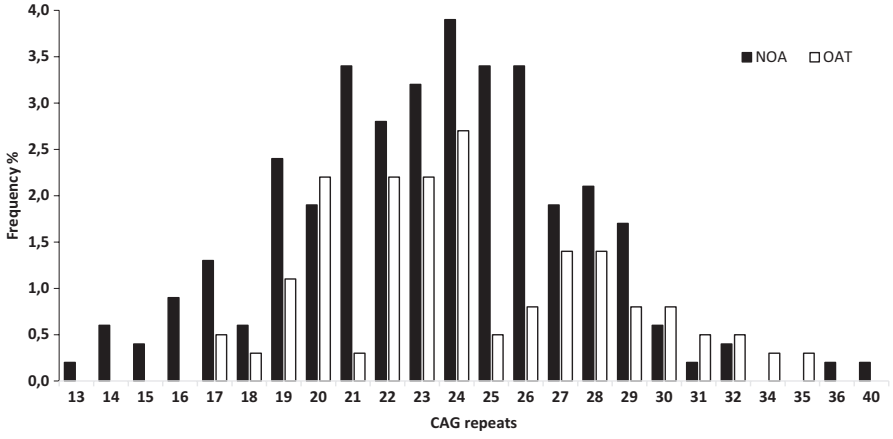

FIGURE 2 Distribution pattern of AR CAG repeats in infertile men with non-obstructive azoospermia (NOA) ($n = 168$) and with oligoasthenoteratozoospermia (OAT; $n = 69$). There were 22 CAG alleles ranging from 13 to 40 repeats in infertile men with NOA, and 18 CAG alleles ranging from 17 to 35 repeats in infertile men with OAT

TABLE 1 Distribution of genotype and allele frequencies of V89L and -308G/A polymorphisms in infertile and fertile men

Genotypes	Infertile patients $n = 312$ (%)	Fertile controls $n = 113$ (%)	OR (95% CI)	*p*-value
SRD5A2				
VV	170 (54.5)	56 (49.5)	Reference	
VL	123 (39.4)	48 (42.5)	1.185 (0.755–1.858)	0.516
LL	19 (6.1)	9 (8)	1.438 (0.615–3.360)	0.711
V	0.74	0.71		
L	0.26	0.29		0.460
TNF-α				
GG	266 (85.3)	95 (84.1)	Reference	
GA	43 (13.2)	17 (15.0)	1.107 (0.602–2.034)	0.856
AA	3 (1.0)	1 (0.9)	0.933 (0.096–9.082)	0.994
G	0.92	0.92		
A	0.08	0.08		0.743

Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 2 Distributions of genotype and allele frequencies in idiopathic infertile men with NOA and OAT

Genotypes	NOA $n = 168$ (%)	OAT $n = 69$ (%)	OR (95% CI)	*p*-value
SRD5A2				
VV	93 (55.4)	36 (52.2)	Reference	
VL	64 (38.1)	30 (43.5)	1.211 (0.678–2.162)	0.620
LL	11 (6.5)	3 (4.3)	0.705 (0.186–2.673)	0.891
V	0.74	0.74		
L	0.26	0.26		0.517
TNF-α				
GG	146 (86.9)	58 (84.1)	Reference	
GA	19 (11.3)	11 (15.9)	1.457 (0.653–3.251)	0.603
AA	3 (1.8)	0 (0)	0.358 (0.018–7.034)	0.723
G	0.93	0.92		
A	0.07	0.08		0.358

Abbreviations: CI, confidence interval; NOA, non-obstructive azoospermia; OAT, oligoasthenoteratozoospermia; OR, odds ratio.

spermatogenesis through the genes involved in this process and/or an alteration in the function of AR, culminating in male infertility. Our results are in contradiction with the studies that could not find any significant association between CAG repeat polymorphism and male infertility (Batiha et al., 2018; Khatami et al., 2015) and this may result from ethnic differences of the study population. Other possible reasons for the contradictory result might be the variety of the infertile subgroups such as azoospermia and oligozoospermia as well as the sample size. A study carried out in Turkish men by Tufan et al. (2005) suggested that idiopathic infertile men including azoospermic, severe oligozoospermic and normozoospermic men had similar CAG repeat length in the fertile controls (Tufan et al., 2005). The distinct result of that study might be attributed to a relatively small study population ($n = 47$ infertile men and $n = 32$ controls) compared to our study which included 113 fertile and 312 infertile subjects. Similarly, a recent study has indicated no association between CAG repeat expansion and NOA in Jordanian men (Al Zoubi et al., 2020).

In our study, short (≤22 CAG) and long CAG (>22 CAG) repeat groups showed no significant difference in the infertility risk. On the

other hand, Delli Muti et al. (2014) suggested that the frequency of short CAG repeat (≤22 CAG) is significantly different between oligozoospermic and normozoospermic men. The authors, however, implicated that there is no association between the seminal parameters and short/long CAG repeats (delli Muti et al., 2014). Variations among the cut-off value for the determination of the subgroups (based on the mean, median or *SD*) as short or long CAG groups may be one of the causes of incompatible findings.

The association of CAG repeats with the serum levels of LH, FSH, testosterone and testicular volume demonstrates contradictory results in several studies, most of which did not find a significant correlation between several hormones and CAG repeats (Badran et al., 2009; Delli Muti et al., 2014; Ma et al., 2014; Sasagawa et al., 2001). In our study, we also could not find any correlations between the CAG repeat expansion and levels of testosterone, LH and FSH. CAG repeat expansion may reduce the transactivation activity of AR via impairment of the functional interaction between AR and coactivators (p160) in the presence of physiological hormone levels, resulting in male infertility.

Androgen actions are regulated by *SRD5A2* polymorphisms and polymorphic *AR* CAG repeat stretches (Moura-Massari et al., 2016). Melting polymorphisms in the *SRD5A2* gene have been identified in men with oligozoospermia or azoospermia (Hines et al., 1999).

In the literature, only three studies investigated the associations among *SRD5A2* gene polymorphisms, semen quality and male infertility in Estonian, Chinese and Turkish men (Peters et al., 2010; Serdarogullari et al., 2021; Zhao et al., 2012). In the Estonian study population, the genotype frequencies of five single nucleotide polymorphisms (SNP) (rs632148, rs523349, rs2300701, rs2268797 and rs12470143) in the *SRD5A2* gene were compared between infertile men with idiopathic azoospermia or oligozoospermia and normozoospermic men. In normozoospermic men, a significant association between the certain alleles of rs632148 and rs12470143 and testicular volume was reported. However, no significant associations were found between the genotypes of *SRD5A2* gene and infertility risk (Peters et al., 2010). In Chinese men, Zhao et al. (2012) reported that the genetic variants of *SRD5A2* had an adverse effect on semen quality (Zhao et al., 2012). In this study, we investigated the possible association of *SRD5A2* V89L polymorphism in idiopathic infertility in Turkish men. Our results show no significant difference between idiopathic infertility and V89L polymorphism in the study population and between oligoasthenoteratozoospermic and nonobstructive azoospermic men. Similarly, Serdarogulları et al. (2021) did not found an association between SRD5A2 V89L polymorphism and NOA in Turkish patients (Serdarogullari et al., 2021). No relationship(s) between the levels of testosterone, LH and FSH, smoking status and *SRD5A2* V89L polymorphism was observed in this study.

TNF-α -308G/A is the promoter polymorphism of the *TNF-α* and has unfavourable effects on sperm parameters (Kurz et al., 2008; Perdichizzi et al., 2007; Shukla et al., 2013; Zalata et al., 2013). The *TNF-α* AA genotype (-308G/A) is more often detected in infertile men than in fertile men and cause a decrease in acrosin activity and seminal α-glycosidase as compared to the GG genotype (Shukla et al., 2013; Tronchon et al., 2008; Zalata et al., 2013). The *TNF-α* A allele is significantly related to azoospermia, oligozoospermia and teratozoospermia but not asthenoteratozoospermia (Bami et al., 2017). Our study suggests no significant association between male infertility and -308G/A polymorphism in Turkish men. Furthermore, infertile men with OAT and NOA displayed similar patterns of genotype and allele frequencies of the *TNF-α* gene.

It is crucial to state that this study has several limitations. Since male infertility is a highly heterogeneous and complex condition and despite our strict inclusion criteria, our study group may still be heterogeneous with small subgroups of qualitative and quantitative spermatogenic impairments. In addition, for a multifactorial disorder, the study population was still relatively small. It was also not possible to obtain data regarding hormonal variables and smoking status from all participants. Therefore, the statistical association between these parameters and infertility may not exactly be reflected.

In conclusion, the results of this study indicate that the *AR* CAG repeat expansion may be one of the risk factors for idiopathic infertility in the Turkish men. However, further studies investigating the association of male infertility with *AR* CAG, V89L and -308G/A polymorphisms are warranted to understand the possible associations among them.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the finding of this study are available from the corresponding author (SG), upon reasonable request.

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