Possible lack of association between E469K polymorphism of ICAM-1 and non-obstructive azoospermia in south-east Turkey

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ABSTRACT
Intercellular adhesion molecule-1 (ICAM-1), a member of the large immunoglobulin superfamily of cell adhesion molecules, is a constituent component of the blood–testis barrier, and it plays a significant role in the homeostasis of spermatogenesis. The E469K polymorphism in the human ICAM-1 gene has been previously associated in various inflammatory/autoimmune disorders. However, the role of the ICAM-1 E469K polymorphism in spermatogenesis remains unclear. The aim of the present study is to analyse the possible association between the ICAM-1 E469K polymorphism and male infertility with non-obstructive azoospermia (NOA) patients within a group of men from Turkey. We included 111 infertile male with NOA and 114 fertile male as control subjects to the study. Genotyping was made by polymerase chain reaction–restriction fragment length polymorphism. The frequency of the genotype and the allele of ICAM-1 E469K were not significantly different between the control group and the patients (P > 0.05). This is the first study to investigate the role of the ICAM-1 gene polymorphism in male infertility with NOA. We conclude that the E469K polymorphism of ICAM-1 is not a risk factor for NOA in the Turkish population.

Introduction
Infertility is a worldwide reproductive health problem defined as the inability to conceive after one year of regular unprotected intercourse, and it affects 10%–15% of all couples worldwide. Male factors account for approximately 50% of all infertility cases, and about 60%–75% of these cases are still unexplained or idiopathic. The most common cause of idiopathic male infertility is impaired spermatogenesis, in which azoospermia is present in about 10%–15% of infertile men [1,2]. Azoospermia is defined as the absence of sperm in semen, and can be typically divided into two major categories: obstructive azoospermia and non-obstructive azoospermia (NOA) in which the testicle fails to produce mature sperm in the ejaculate [2].

There are numerous causes of NOA, ranging from hormonal, environmental/lifestyle factors, reactive oxygen species, epigenetic abnormalities and genetic factors, such as the mutations of chromosomal, gene and mitochondrial DNA [1,2]. Yq microdeletions are the best-known gene mutations leading to impaired spermatogenesis, which may involve several genes within the azoospermia factor region. Moreover, a number of mutations of novel or known autosomal genes have been considered to be associated with male infertility in humans. These genes include TAF4B and ZMYND15, SYCP3, NRSO1, MTHFR, SHBG, FSHR, YBX2 and ART3 as well as TAF7 L on the X-chromosome [2]. Despite the large number of genes known to contribute to impaired spermatogenesis, the total contribution of these genes to the condition is very small [2].

During spermatogenesis, Sertoli–Sertoli and Sertoli–germ cell junctions have an important role in germ cell movement in the seminiferous epithelium. For spermatogenesis to reach completion, restructuring takes place at these junctions because developing germ cells are non-motile, and must move ‘up and down’ the seminiferous epithelium to accommodate the cellular events of mitosis, meiosis, spermiogenesis and spermiation [3–5]. Furthermore, preleptotene spermatocytes at the basal compartment must traverse the blood–testis barrier (BTB), also known as the Sertoli cell barrier, to enter the adluminal compartment. Thus, it is indispensable that restructuring and turnover take place at the Sertoli cell–cell and Sertoli–germ cell junctions during spermatogenesis [3–5]. If germ cell adhesion were to be...
One hundred and eleven infertile men with NOA were recruited in this study. Only idiopathic infertile men with NOA, with at least one year of infertility, were included. Individuals with known causes of infertility, including genetic factors (chromosome anomalies, azoospermia factor [AZF] microdeletions), lifestyle factors (e.g. alcoholism and occupation), clinical factors affecting the fertility (drug intake, infections, hereditary hypothalamic-pituitary abnormalities, varicocele and cryptorchidism, etc.) and men whose partner had factors involved in infertility were excluded from this study. The control group was selected from healthy men who have had fathered at least one healthy child within one year without assisted reproductive measures. Both the men participating in the infertility workup as well as the fertile controls were recruited within the same geographical region.

All studied fertile and infertile men were referred from the Urology Department of Dicle University Hospital to the Medical Biology and Genetics Department. The study was approved by the Ethics Committee of Dicle University’s Faculty of Medicine and all participants gave their informed consent (87/26.02.2016).

**SNPs selection and genotyping of ICAM-1 gene polymorphisms**

In the present study, we selected one non-synonymous SNP, 1462A>G (E469K; rs5498) of the ICAM-1 gene for genotyping [10]. This polymorphism is located in Exon 6 of the ICAM-1 gene. The SNP ID number and detailed sequence information is available in the public SNP database (http://www.ncbi.nlm.nih.gov/SNP/).

Heparinised peripheral venous blood (2 mL) was collected from each subject and stored in tubes containing ethylenediaminetetra acetic acid at −20 °C until the DNA extraction step. Genomic DNA was extracted from whole blood by salting out [14].

Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to determine the SNP with appropriate primer sets and restriction enzyme as previously described by Miller et al. [15]. The primer sets and enzymes used in this study are shown in Table 1.

The PCR reaction was carried out in a 20 µL reaction volume containing 1 x PCR buffer, 2 mmol/L MgCl₂,
**Results and discussion**

Although association of the ICAM-1 E469K polymorphism with several diseases has been previously shown [7], as yet, there is no final conclusion about the association of this polymorphism with diseases. For example, Jiang et al. [16] explored the possible role of the ICAM-1 E469K polymorphism in the susceptibility to CHD and myocardial infarction and found that GG and AG genotypes were possibly associated with susceptibility to these conditions in Germans. Similar results have been demonstrated by several studies in the Egyptian and Chinese population [17,18]. In a meta-analysis study, Ji et al. [8] found that the ICAM-1 E469K polymorphism is associated with CHD risk and the G allele is a more significant risk factor for developing CHD among Asian and Caucasian populations. However, Mo et al. [19] found that the A allele of ICAM-1 E469K might be a genetic risk factor for CHD. In other reports, it is demonstrated that this SNP of the ICAM-1 gene is associated with Behcet’s disease in Japanese patients [6], type 1 diabetes in a Swedish population [10] and colorectal cancer in a Chinese population [11]. In contrast, there are also results that report no association between this SNP and diseases, such as ischemic heart disease in the Irish population [20], acromegaly in the Turkish population [7], celiac disease in the north Indian population [21] and non-diabetic metabolic syndrome in the Turkish population [22].

In the present study, the E469K (rs5498) polymorphism in the ICAM-1 gene was identified in the patients with NOA and the control subjects within a Turkish population. The genotype and allele frequencies of this SNPs among the infertile men and fertile controls are summarized in Table 2. To the best of our knowledge, the present study is the first to investigate the association between the ICAM-1 E469K polymorphism and the risk of developing NOA. We found no association between the ICAM-1 E469K polymorphism and NOA in the Turkish population ($P > 0.05$). However, further analysis needs to cover a much larger population or other ethnic groups as well.

The heterozygosity index for rs5498 in the genotype distribution of this population was high both in the patients and the controls: 53.15% and 44.74%, respectively.
respectively (Table 2). These results show similarity with our previous study on the role of this polymorphism in non-diabetic metabolic syndrome in the Turkish population, in which the results did not show any associations between this polymorphism and disease [22]. In addition, the high frequency of the heterozygous genotype for ICAM-1 E469K in the present study is also in accordance with that reported in other previous studies on Turkish, Danish, Finnish, Japanese and Swedish populations [10]. Although our study had a limited number of patients, we could speculate that this situation may reflect that there may be a common ancestor in these populations.

ICAMs are cell–cell and cell–extracellular matrix adhesion receptors that are known to be expressed in a number of epithelial and endothelial cells, leukocytes, platelets, erythrocytes, macrophages, dendritic cells, fibroblasts, some tumour cells, as well as testicular cells [4,5]. It is reported that ICAMs are crucial regulatory molecules of spermatogenesis. ICAM-1 and its biologically active soluble fragment (sICAM-1) generated from the proteolytic cleavage of the ICAM extracellular domain, suggesting that it may be critical for adhesion as non-motile germ cells traverse the ‘opened’ BTB. It is reported that these regulatory molecules (e.g. inflammatory mediators such as TNF-α) are needed to induce cleavage of the ICAM extracellular domain. Thus, the loss and/or defects of these molecules may cause infertility in animals and humans [3–5,23–26]. Although, the data from immunohistochemical studies demonstrated the role of the ICAM genes on spermatogenesis, there is no molecular genetic study to explain the role of ICAM-1 gene defect in spermatogenesis in both animals and humans. To our knowledge, this is the first study to investigate the association of this polymorphism with idiopathic infertility in men. Such studies will unravel whether defects in the gene also lead to infertility in human males.

Previous in vivo and in vitro studies have shown that the pro-inflammatory cytokines (such as TNF-α and interleukin-1α) and several ICAMs are significant regulators of Sertoli–Sertoli/BTB and Sertoli–germ cell junctions, and differentially expressed during spermatogenesis. Barrier restructuring correlates with ICAM-1 and its soluble forms (sICAM-1), generated from the proteolytic cleavage of the ICAM extracellular domain, suggesting that it may be critical for adhesion as non-motile germ cells traverse the ‘opened’ BTB. It is reported that these regulatory molecules (e.g. inflammatory mediators such as TNF-α) are needed to induce cleavage of the ICAM extracellular domain. Thus, the loss and/or defects of these molecules may cause infertility in animals and humans [3–5,23–26]. Although, the data from immunohistochemical studies demonstrated the role of the ICAM genes on spermatogenesis, there is no molecular genetic study to explain the role of ICAM-1 gene defect in spermatogenesis in both animals and humans. To our knowledge, this is the first study to investigate the association of this polymorphism with idiopathic infertility in men. Such studies will contribute substantially for the identification of genes involved in spermatogenesis. Future studies might unravel whether defects in the gene also lead to infertility in human males.

### Conclusions

This study showed that the ICAM-1 E469K polymorphism was not involved in the susceptibility to NOA in the studied cohort. More comprehensive studies within larger patient groups, preferably involving multiple ethnicity and multiple centres, are required to explain any potential role of the ICAM-1 E469K polymorphism as a male infertility risk factor in NOA.

### Disclosure statement

The authors declare that there are no conflicts of interest.

### Table 2. Genotype and allele frequencies of the ICAM-1 gene among patients with idiopathic azoospermia and fertile controls.

| Genotypes | Patients (n = 111) | Controls (n = 114) | OR | 95% CI | P-value |
|-----------|------------------|------------------|----|--------|---------|
| Genotype  | n                | %                | n  | %      |         |
| AA        | 30               | 27.03            | 42 | 36.84  | 1       |
| AG        | 59               | 53.15            | 51 | 44.74  | 1.62    | 0.88–2.95 | 0.11 |
| GG        | 22               | 19.82            | 21 | 18.42  | 1.46    | 0.68–3.13 | 0.32 |
| Allele    |                  |                  |    |        |         |         |
| A         | 119              | 53.61            | 135| 59.21  | 1       |         |
| G         | 103              | 46.39            | 93 | 40.79  | 1.25    | 0.86–1.82| 0.23 |

Note: Control and patient group consistent with Hardy–Weinberg equilibrium (HWE). $X^2 = 1.45, P = 0.22$ and HWE $X^2 = 0.52, P = 0.469$, respectively. OR, odds ratio; CI, confidence interval.
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