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NEXT-GENERATION SEQUENCING INFERTILITY PANEL IN TURKEY: FIRST RESULTS

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ABSTRACT

Background

Male infertility is a complex pathophysiological disorder. At least 2000 genes are implicated in the etiology of male infertility, making it a very complex genetic condition. In cases of male infertility, genetic testing using next-generation sequencing (NGS) technology may be useful for diagnosis. Thus, the purpose of this investigation was to apply the diagnostic offer for genetic variant identification using an NGS panel.

Methods

We developed an NGS gene panel that we used in 85 infertile male patients. The panel consisted of 132 genes exploring the genetic causes of male infertility; namely spermatogenesis failure due to single-gene mutations, central hypogonadism, androgen insensitivity syndrome, congenital hypopituitarism, and primary ciliary dyskinesia etc.

Results

A total of 85 patients (85 males) between 21 year and 45 years old were included in the study group. NGS analysis had been applied in all the primary infertility cases. As a result of NGS analysis, 58 clinical variants in 28 genes were detected in 41 patients (%48.23- 41/85)

Conclusion

Consequently, pre-diagnostic genes included in a custom-made NGS panel test can enhance genetic diagnostic testing and have an impact on the clinical management of male infertility.

Keywords: Male infertility; NGS diagnosis; Genetic causes of male infertility

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INTRODUCTION

The way that male infertility is treated has undergone a significant transformation as a result of our growing understanding of the physiology of male reproduction, fertilization, and the development of increasingly potent assisted reproductive procedures. A physical exam and medical history gathering are currently part of the diagnostic procedure provided to infertile male patients. This is followed by a mix of laboratory tests specifically chosen for each case, including a thorough genetic laboratory analysis. At least a year of infertility should precede the administration of diagnostic testing. Accordingly, a couple is considered infertile if they are unable to conceive following a year of regular, unprotected sex. 15% of male patients who are infertile have genetic issues. Chromosome abnormalities or single gene mutations are examples of them. The Online Mendelian Inheritance in Man (OMIM) database contains information on more than 200 genetic conditions associated with male infertility (1-4).

Many disorders, most notably Mendelian or uncommon diseases where having causal variants significantly reduces reproductive fitness, have had exceptional results using NGS (5).

The candidate gene approach in model animals and whole genome investigations using single-nucleotide polymorphism microarray and next-generation sequencing (NGS) technologies, such as exome or whole-genome sequencing, are the two main methods for identifying the genes responsible for infertility. The reason of male infertility is still unknown in up to 70% of instances, despite extensive diagnostic testing, because traditional genetic tests sometimes fall short of making a diagnosis. Recent studies appear to address how NGS technology is increasing the rate of male infertility diagnosis. Accordingly, it has already been established that several diagnostic genes have a role in the pathophysiology of male infertility. It may be possible to make a diagnosis with the use of pre-

diagnostic genes, such as those that have been linked to male infertility but do not yet have solid proof of a causal relationship (6, 7, 8).

To do this, the current study was conducted to assess a number of pre-diagnostic genes by contrasting the outcomes with those obtained using our standard NGS custom-made gene panel for the diagnosis of male infertility, which consists of 132 genes. The genes included in the gene panel are composed of genes that have been associated with infertility to date.

METHODS

Patients and Samples

The research included 85 individuals with a clinical diagnosis of male infertility who had tested negative on diagnostic genetic testing. 84 individuals were thought to have primary spermatogenic failure, while one individual was thought to have central hypogonadism. Following the elimination of female factor infertility and acquired reasons of male infertility, main spermatogenic failure was suspected with a history of couple infertility longer than two years (e.g. male accessory gland infection, varicocele, testicular trauma, etc.). All patients are cases of infertility for two or more years. The patient group consists of patients who do not have any known additional disease or malignancy.

Additionally, individuals included in this study tested negative for early genetic anomalies such karyotype abnormalities, Y chromosome AZF microdeletions. MLPA technique was performed using the SALSA MLPA probemix P360 version B1 (MRC Holland, Amsterdam, The Netherlands) kit following the manufacturer's instructions. The kit contained 55 probes, of which 12 were located in autosomal chromosomes (for internal control reaction), and 43 were located in Y-chromosome AZF regions (16 AZFa, 15 AZFb, and 12 AZFc regions).

Each patient provided written consent after being fully briefed. The study was conducted in accordance with the principles outlined in the Declaration of Helsinki, and the local Ethics Committee approved the study.

Conventional G-banded karyotype analysis from peripheral blood was performed as part of the initial screening tests. The study included peripheral lymphocyte culture by a standard method using the Leishman-banding technique, centromere-banding (C-banding) and nucleolar organizing region staining performed as needed according to the AGT Cytogenetics Laboratory Manual. The best metaphases were karyotyped, and the total chromosome count was usually determined in 25 cells. The International System for Human Cytogenetic Nomenclature (ISCN) was used for the nomenclature of human chromosomes. Patients with no anomalies as a result of karyotype analysis were included in the NGS analysis.

Eighty five samples were sequenced using QIAseq Targeted DNA Custom Panel (Qiagen, Hilden, Germany). 2 mL of peripheral blood were collected and then preserved in anticoagulation tubes. Genomic DNA was isolated from peripheral whole blood using the EZ1 DNA Investigator Kit (Qiagen, Hilden, Germany). After DNA extraction, target sequences were enriched by using customized capture probes chips (Illumina, San Diego, CA). This kit included 132 genes targeting disease. Libraries covering the target genes were prepared according to the QIAseq Targeted DNA Panel protocol (Qiagen, Hilden, Germany). Following the target enrichment process, libraries were sequenced on the MiSeq System (Illumina, San Diego, CA, USA). OCI analysis (Qiagen, Hilden, Germany) was used for Quality control and Variant Call Format file generation. In silico evaluation of the pathogenicity of nucleotide changes in exons was performed using Polymorphism Phenotyping v2 (PolyPhen-2, http:// genetics.bwh.harvard.edu/pph2/), Sorting Intolerant from Tolerant (SIFT, https://sift.bii.a-star.edu.sg/), and MutationTaster (http://www.mutationtaster.org). Minor allele frequencies (MAF) were checked in the Genome Aggregation Database gnomAD (http://gnomad.broadinstitute.org/).Variant analysis was performed with Ingenuity software (Qiagen, Hilden, Germany). Variants were interpreted according to the American College of Medical Genetics and Genomics (ACMG) recommended standard. Sanger sequencing was performed for confirmation when the target region coverage was less than 15 reads. Nucleotide alterations were analyzed and validated by Sanger sequencing. After confirmation, each variant was classified as a pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign, or benign, according to the American College of Medical Genetics (ACMG) guidelines. Coding genomic regions (CDS) that were sequenced with coverage less than 15X were eventually re-sequenced using Sanger technology.

RESULTS

A total of 85 patients (85 males) between 21 years and 45 years old were included in the study group. NGS analysis had been applied in all the primary infertility cases. As a result of NGS analysis, 58 clinical variants in 28 genes were detected in 41 patients (%48,23- 41/85) (Table 1). Thirty-two of these variants are unknown clinical significance (VUS), 11 of them likely pathogenic, and 15 of these variants are classified as pathogenic in according to the Varsome, The Human Genomic Variant Search Engine, Franklin by Genoox, Clinvar and American College of Medical Genetics and Genomics (ACMG) databases.

Table 1. NGS panel for the diagnosis of male infertility

1.	NPHP4	23.	DAZL	45.	FKBPL	67.	DDX25	89.	ZMYND15	111.	SYCP2
2.	C1orf167	24.	DNAH1	46.	PLG	68.	C1RL	90.	GP1BA	112.	CBS
3.	MTHFR	25.	PROS1	47.	ZPBP	69.	C1RL-AS1	91.	KLHL10	113.	DNMT3L
4.	CLCA4	26.	BOC	48.	C7orf61	70.	DPY19L2	92.	ITGB3	114.	POFUT2
5.	BRDT	27.	CFAP44	49.	SERPINE1	71.	CHPT1	93.	TEX14	115.	GP1BB
6.	F3	28.	CFAP44-AS1	50.	POLR2J3	72.	SYCP3	94.	ACE	116.	POLR2F
7.	SPAG17	29.	GP9	51.	CFTR	73.	CCDC62	95.	PGS1	117.	SOX10
8.	F5	30.	SPATA16	52.	TEX15	74.	PIWIL1	96.	DNAH17	118.	PICK1
9.	SERPINC1	31.	CCDC39	53.	PLAT	75.	CPB2	97.	DNAH17-AS1	119.	MEI1
10.	F13B	32.	GP5	54.	CHD7	76.	F7	98.	TAF4B	120.	ADGRG2
11.	MTR	33.	CEP135	55.	TMEM70	77.	F10	99.	GGN	121.	MAGEB4
12.	LHCGR	34.	SPINK2	56.	CCIN	78.	TDRD9	100.	PLAUR	122.	MAGEB1
13.	FSHR	35.	BMP3	57.	NR5A1	79.	CATSPER2	101.	LHB	123.	NR0B1
14.	DNAH6	36.	FGB	58.	ASS1	80.	TERB2	102.	NLRP7	124.	TBC1D25
15.	NPAS2	37.	FGA	59.	UPF2	81.	NME4	103.	NLRP2	125.	AR
16.	LOC101927142	38.	KLKB1	60.	CFAP43	82.	FAHD1	104.	AURKC	126.	TEX11
17.	PROC	39.	F11	61.	NANOS1	83.	MEIOB	105.	SIRPG	127.	USP26
18.	TFPI	40.	MTRR	62.	SYCE1	84.	SEPT12	106.	SIRPA	128.	F9
19.	STRADB	41.	PRDM9	63.	FSHB	85.	PRM1	107.	THBD	129.	F8
20.	C2CD6	42.	ITGA2	64.	F2	86.	TERB1	108.	SUN5	130.	FUNDC2
21.	LOC100129175	43.	F2R	65.	MAJIN	87.	SERPINF1	109.	E2F1	131.	SRY
22.	CFAP65	44.	F13A1	66.	CATSPER1	88.	CXCL16	110.	PROCR	132.	USP9Y

The most frequently observed variants are those observed in the CFTR gene. 18 CFTR gene variants were detected in 16 different patients. Among these, 7 variants are pathogenic, 4 variants are likely pathogenic and 7 variants are VUS. The remaining 40 variants are distributed among the other 27 genes in the panel. Among these, 8 variants were evaluated as pathogenic, 7 variants as likely pathogenic, and 25 variants as VUS. Segregation analyses could not be performed in patients with VUS. Pathogenic and likely pathogenic variants were detected de novo. Among the variants considered as VUS, the most frequently observed variants clustered in the *DNAH1* gene. Among the pathogenic variants, the most common variants after *CFTR* were detected in the *CBS* and *F11* genes (Table 2). We also detected a compound heterozygous *CFTR* variant in one of our patients. This condition of the patient was evaluated clinically.

Table 2. Variants detected in the patient group as a result of NGS

Patient n.	VUS - inheritance	Likely pathogenic- inheritance	Pathogenic- inheritance
1.	NM_000130.5(F5):c.1128G>T p.R376S **(PP3) (AD,AR)** NM_001370.2(DNAH6):c.8422G>A (p.V2808I) **(PP3)** **(n/a)**		
2.	NM_000313.4(PROS1): c.1021G>T (p.A341S) **(PM1,PM2,PP3) (AD)**	NM_000492.4(CFTR):c.1516A>G (p.Ile506Val) **(PM1,PM2,PM3,PP3,BP6) (AR)**	
3.	NM_015512.5(DNAH1):c.8885A>C (p.Lys2962Thr) **(PP3) (AR)**		
4.	NM_000492.4(CFTR):c.443T>C (p.Ile148Thr) **(PS3,M1,PM2,PM3,PP3,BS2, BS3,BP2,BP6) (AR)**		

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5.	NM_000492.4(CFTR):c.2981T>G (p.Phe994Cys) **(PM1,PM2,PP3) (AR)**		NM_000128.4(F11):c.1556G>A (p.Trp519Ter) **(PVS1,PS4,PM2,PM3,PP5)** NM_000128.4(F11):c.403G>T (p.Glu135Ter) **(PA1,PVS1,PS3,PS4,PM3,PP5,BS1,BS2) (AD,AR)**
6.	NM_001242805.2 (BRDT):c.163C>T (p.Pro55Ser) **(PP3) (AR)**		
7.	NM_001350162.2 (TEX15):c.2580_2583del (p.Asp860GlufsTer15) **(PS3,PP3) (AR)**	NM_000173.7(GP1BA): c.1235_1298delAGCCCAC... (p.E412fs*39) **(PVS1,PM2) (AD,AR)**	
8.	NM_000789.4(ACE):c.2299G>A (p.Glu767Lys) **(PM2,PP3) (AR)**		
9.		NM_000312.4(PROC):c.982C>T (p.Arg328Cys) **(PM1,PM2,PP3,PP5) (AD,AR)**	NM_002203.4(ITGA2):c.981_985del (p.Lys327AsnfsTer6) **(PVS1,PM2) (n/a)**
10.			NM_000492.4 (CFTR):c.2491G>T (p.Glu831Ter) **(PVS1,PS3,PS4,PM2) (AR)**
11.			NM_000492.3(CFTR):c.1521_1523del (p.Phe508del) **(PA2,PS3,PM1,PM4,PP3,BS3, BS4,BP2,BP5) (AR)**
12.			NM_000492.4(CFTR):c.1210-11T>G **(PA2,PS3,PM3,PP5,BS1,BS2,BP2) (AR)**
13.	NM_000492.4(CFTR):c.2991G>C (p.Leu997Phe) **(PS1,PM1,PM2,PM3,PP3,BS2,BP2,BP6) (AR)**		
14.			NM_054012.4(ASS1):c.535T>C (p.Trp179Arg) **(PS3,PS4,PM1,PM2,PM3,PP3,PP5) (AR)**
15.	NM_144605.4(SEPT12): c.208T>C (p.Phe70Leu) **(PM2,PP3)**	NM_000492.4(CFTR): c.1397C>T (p.S466L) **(PM1,PM2,PM3,PP3) (AR)**	
16.	NM_000492.4(CFTR):c.2973A>G (p.Ile991Met) **(PM1,PM2) (AR)**		
17.	NM_015512.5(DNAH1):c.10164G>T (p.K3388N)**(PP3) (AR)**		
18.		NM_015102.5(NPHP4):c.224G>A (p.Trp75Ter) **(AR)**	
19.	NM_000492.4(CFTR): c.1043T>A (p.M348K) **(PM1,PM2,PM3,PP3,BP2,BP6) (AR)** NM_012128.4 (CLCA4): c.760dupA (p.T254fs*3) **(PVS1) (n/a)**	NM_000492.4 (CFTR) : c.3038C>T (p.P1013L) **(PM1,PM2,PM3,PP3)**	
20.			NM_000071.3(CBS):c.833T>C (p.Ile278Thr) **(PS3,PS4,PM1,PM2,PM3,PM5,PP3) (AR)**
21.			NM_000128.4(F11):c.325G>A (p.Ala109Thr) **(AD,AR) (PS3,PS4,PM1,PM2,PM3,PP3,PP5)** NM_000492.4(CFTR):c.1516A>G (p.Ile506Val) **(PM1,PM2,PM3,PP3,BP6) (AR)**
22.		NM_173812.5(DPY19L2): c.247C>T (p.Q83*) **(PVS1,PM2) (AR)**	NM_000492.4(CFTR):c.1521_1523delCTT (p.F508del) **(PA2,PS3,PS4,PM1,PM3,PM4, PP3,PP5,BS1,BS2,BS4,BP2) (AR)**
23.		NM_000492.3(CFTR):c.3872A>G (p.Q1291R) **(PM1,PM2,PM3,PM5,PP3,PP5) (AR)**	
24.			NM_000071.3(CBS):c.833T>C (p.I278T) **(PS3,PS4,PM1,PM2,PM3,PM5,PP3) (AR)**
25.	NM_000492.4(CFTR):c.3256A>G (p.Thr1086Ala)**(PP3) (AR)**		

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26.	NM_000894.2 (LHB):c.169T>C NP_000885.1:p.Tyr57His **(PM2,PP3) (AR)**		
27.		NM_000301.5(PLG):c.2384G>A (p.Arg795His) **(PS4,PM2,PP3) (AD,AR)**	
28.	NNM_000789.4(ACE): c.3490G>A (p.G1164R) **(PM2,PP3) (AR)**		
29.			NM_000492.4(CFTR): c.350G>A (p.R117H) **(PA2,PS3,PM1,PM2,PM3,PM5,PP1,PP3, PP5,BS2,BS4,BP2) (AR)**
30.	NM_015512.5 (DNAH1): c.8976C>G p.F2992L**(PP3) (AR)**		
31.		NM_000131.4 (F7): c.805+3_805+6delGGGT (-) **(PVS1,PM2) (AR)**	
32.	NM_001994.3(F13B): c.209A>C (p.Q70P) **(PM2,PP3) (AR)**		NM_000492.4 (CFTR) : c.1521_1523delCTT (p.F508del) **(PA2,PS3,PM1,PM3,PM4,PP3,PP5,BS1, BS2,BS3,BS4,BP2) (AR)**
33.		NM_017780.4 (CHD7) :c.5995G>A (p.A1999T)**(PS4,PM2,PP2,PP3) (AD)**	
34.	NM_012128.3 (CLCA4):c.575C>A NP_036260.2:p.Ser192Cys **(PP3) (n/a)**		
35.	NM_015512.5(DNAH1): c.9495G>A (p.Thr3165) **(PM2,PP3) (AR)** NM_152467.5 (KLHL10): c.1226A>G (p.E409G) **(PM2,PP3) (AD)** NM_144605.4 (SEPTIN12) :c.611G>T (p.Arg204Leu) **(PP3) (AD)**		
36.	NM_001330438.2 (DDX25): c.110C>T (p.Ala37Val) **(PP3) (n/a)**		
37.			NM_000071.3(CBS):c.833T>C (p.Ile278Thr) **(PS3,PM1,PM5,PP3)**
38.	NM_001312675.1 (F10):c.202C>T NP_001299604.1:p.Arg68Cys **(PM2,PP3) (AR)** NM_000212.2 (ITGB3):c.1576G>C NP_000203.2:p.Glu526Lys **(PP3) (AD,AR)** NM_015512.5 (DNAH1): c.4642C>G (p.L1548V) **(PM2,PP3) (AR)**		
39.	NM_000301.5(PLG):c.2134G>A (p.Gly712Arg) **(PP3) (AD,AR)**		
40.	NM_000492.4(CFTR):c.890G>A (p.Arg297Gln) **(PS3,PM1,PP3,BS3,BS6) (AR)** NM_173812.5(DPY19L2): c.2221C>A (p.P741T) **(PM2,PP3) (AR)**		
41.	NM_173628.3(DNAH17):c.7752+2T>A **(PVS1) (AR)**		
Total	**32**	**11**	**15**

AD: autosomal dominant; **AR:** autosomal recessive

DISCUSSION

Highly diverse phenotypic representation and a complicated multifactorial etiology, including environmental and genetic factors, characterize the condition of male infertility. In most cases, it is challenging to identify a genetic cause of infertility due to the large number of candidate genes (9, 10). In any case, a multi-disease gene panel can help identify the cause of male infertility. In order to categorize genetic variants, a multifactorial likelihood model can be used to assess the likelihood that a variant is pathogenic based on a previous likelihood of patho-

genicity based on in silico research and the genetic and epidemiological data that are currently available (11-13). Genetic variants can be categorized into five categories according to the American College of Medical Genetics and Genomics' references: pathogenic, likely pathogenic, variant of unknown consequence, likely benign, or benign (14). A genetic alteration known as a VUS has ambiguous effects on gene function. The interpretation of VUS is a challenging task for the clinical management of infertile male patients and genetic counseling. Since VUS are not clearly related with a phenotype currently, but could be categorized as pathogenic in the future, it is crucial to detect and assess them. An example of this situation is the NM_000071.3(*CBS*):c.833T>C (p.Ile278Thr) variant detected in our patient group. This variant, which was evaluated as VUS in the databases at the beginning of our study, was later classified as pathogenic.

Variants in *PROS1* and *CFTR* were detected in our patient number 2. *PROS1* variants show autosomal dominant inheritance. Even though the detected variant was evaluated as VUS, the classification of this variant should be followed in the future.

Similarly, in our patient number 5, 2 pathogenic variants belonging to the F11 gene and a variant evaluated as VUS in *CFTR* were detected. F11 variants show autosomal dominant inheritance. The detected variants were evaluated as pathogenic. OMIM has been associated with Factor XI deficiency.

In another patient, case number 7, variants were detected in 2 separate genes. VUS evaluation was performed for *TEX15*. *TEX15* is associated with the Spermatogenic failure 25 phenotype in the OMIM database, and exhibits an autosomal recessive pattern of inheritance. The other variant detected in the patient is a possible pathogenic variant belonging to the *GP1BA* gene. This gene, which is associated with different types of diseases in the OMIM database, can show autosomal dominant and recessive inheritance. Particularly notable among these diseases are Bernard-Soulier syndrome, type A2 (dominant) and von Willebrand disease, platelet-type (dominant).

PROC variant was detected in patient number 9 and was reported as possibly pathogenic. PROC variants have been associated with autosomal recessive and dominant forms of Thrombophilia 3 due to protein C deficiency in OMIM. Another variant in the patient is the pathogenic variant in the *ITGA2* gene. The inheritance pattern and phenotype of variant of this gene have not yet been elucidated.

We detected variants in *SEPT 12* and *CFTR* genes in patient number 15. *SEPT* 12 variants cause autosomal dominant Spermatogenic failure 10.

In patient number 19, we detected compound heterozygous variants of the *CFTR* gene and an additional variant of the *CLCA4* gene. While *CFTR* gene variants exhibit autosomal recessive inheritance, there are no entries in the databases yet for the *CLCA4* gene.

We detected *F11* and *CFTR* pathogenic variants in patient number 21. *F11* was found to be associated with autosomal dominant and recessive forms of Factor XI deficiency in the OMIM database.

We detected *DPY19L2* and *CFTR* pathogenic variants in patient number 22. *DPY19L2* was found to be associated with autosomal recessive forms of spermatogenic failure 9 in the OMIM database. Similarly, patient number 40 has variants in these two genes that were evaluated as VUS.

F13B and *CFTR* variants were detected in another of our patients, case number 32. *F13B* has been associated with Factor XIIIB deficiency in OMIM. *F13B* variants show autosomal recessive inheritance.

Interestingly, variants in 3 different genes were detected in our last 2 patients. Variants considered to be VUS were detected in the *DNAH1, KLHL10* and *SEPT12* genes in the first patient. *KLHL10* and *SEPT12* variants have been associated with autosomal dominant spermatogenic failure. DNAH1 has been found to be associated with autosomal recessive Ciliary dyskinesia, primary and Spermatogenic failure. These findings were associated with the patient's phenotype. In the second patient, variants considered to be VUS were detected in the *DNAH1, F10* and *ITGB3* genes. The *F10* gene has been associated with autosomal recessive Factor X deficiency. *ITGB3* has been associated with autosomal recessive Bleeding disorder, platelet-type and autosomal dominant Glanzmann thrombasthenia.

Although it is difficult to reconcile those with recessive inheritance in the detected variants with the patient clinics, those with dominant inheritance were compatible with the patient clinics.

DNAH1 gene is the most frequently detected VUS variant in our patient group. A diverse range of patients with aberrant flagellar structures have been shown to have mutant *DNAH1* in the majority of current research. Male infertility has been linked to numerous morphologic defects of the sperm flagella caused by mutations in *DNAH1*. After intracytoplasmic sperm injection, patients with multiple morphologic abnormalities of the flagella (MMAF) caused by mutations in the *DNAH1* gene have a favorable prognosis. These investigations have demonstrated that dysplasia of the sperm fibrous sheath (DFS) and infertility are directly related to abnormalities in the *DNAH1* gene (15-18).

The most frequently detected *CFTR* variants in our patient group were seen in all 3 groups (pathogenic, likely pathogenic, VUS). One of the most researched genes for male infertility, the *CFTR* gene, has 27 exons and more

than 180,000 base pairs of DNA. A membrane ion channel protein called CFTR, which is encoded on chromosome 7p, controls the vas deferens in the male genital tract. Considered a moderate type of cystic fibrosis (CF), CBAVD is a major contributing factor to obstructive azoospermia (OA) and is one of the primary factors leading to male reproductive abnormalities. Previous research has shown a positive correlation between *CFTR* mutations and CBAVD. There are about 1,500 variants listed in the *CFTR* database. Mutations in the F508 and IVS8-5T genes may be important in nonobstructive male infertility disorders such oligozoospermia and nonobstructive azoospermia (NOA). According to our findings, there is a chance that the genetic variant IVS8-5T could serve as a biomarker for nonobstructive male infertility. Three of the pathogenic variants we detected in the patient group are IVS8-5T. We also detected a compound heterozygous *CFTR* variant in one of our patients. The condition of the patient was evaluated clinically. *CFTR* variant rates are also significantly higher than the carrier rate reported in our patient group (19-22).

Another gene we detected among pathogenic gene variants is the *CBS* gene. The *CBS* gene is the most common locus for mutations associated with homocystinuria. Cystathionine-β-synthase, also known as CBS, is an enzyme that is encoded by the CBS gene in humans. The trans-sulfuration pathway enzymes cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) are recognized for their non-specific substrate recognition. These enzymes provide their substrates an alternate CBS and CSE pathway, allowing them to function somewhat in reverse. In addition, these enzymes are involved in the synthesis of hydrogen sulphide (H_2S). This is a gaseous transmitter with antioxidant and anti-inflammatory properties. Although both CSE and CBS are frequently present in the testes—CSE is mostly found in immature germ cells and Sertoli cells, while CBS is extensively distributed in Leydig cells, germ cells, and Sertoli cells—it is unclear how much each kind of cell contributes to the production of H_2S in the testes. Numerous research studies have demonstrated that male infertility is associated with failure of one carbon metabolism, namely the imbalance of CBS and CSE enzymes in the trans-sulfuration pathway, and a specific deficit in H_2S output is documented (23, 24).

Another gene we detected as a pathogenic variant in our patient group was *FXI*. Compound heterozygous *FXI* pathogenic variant was detected in one of our patients. In another patient, we detected a heterozygous *FXI* variant of *CFTR* accompanied by pathogenic variant. *FXI*, or coagulation factor XI, inhibits fibrinolysis and encourages the production of fibrin. One measurement only elevated plasma FXI levels are linked to an increased risk of thrombosis. Hemophilia C, another name for factor XI deficiency, is an autosomal recessive condition mostly affecting Ashkenazi Jews. It is typically linked to variants in bleeding characteristics. The majority of transmission is autosomal recessive. Couples who are considered at-risk (both individuals carry a mutation that causes the disease) should be informed through genetic counseling that there is a 25% chance that each pregnancy will result in a homozygous child who is affected. There have also been reports of heterozygous patients with bleeding symptoms, pointing to an autosomal dominant mode of transmission with varying penetrance (25, 26).

Genes that code for hormones and hormone receptors which are involved in the functioning of the human reproductive system are included in the fertility panel design. Numerous investigations have demonstrated the association between specific polymorphisms in genes encoding receptors, including those that bind to FSH and LH, and the results of an ovarian hyperstimulation cycle under control and in vitro fertilization treatment. The intended genetic panel's results will yield the data required to ascertain the frequency of these variants in our community and assess the panel's usefulness in clinical settings.

The involvement of the clinicians who seek this genetic investigation needs to be emphasized. If the gene panel is able to pinpoint the underlying reason of infertility, clinicians will need to have a comprehensive picture of the patient's phenotype. "Idiopathic infertility" affects a large number of individuals, and while a genetic component may be identified in certain cases, the absence of a distinct phenotype may make it more difficult to interpret the data, particularly variants with unclear significance. Clinicians should also be aware that three factors play a major role in how these investigations are interpreted: the patient's phenotypic characteristics, their medical history, and any pertinent family history. For the diagnostic laboratory to properly interpret variants found through testing, it is imperative that they have information about all observable traits as well as the family's medical history (27-30).

The first unique gene sequencing panel intended for the diagnosis of hereditary infertility in males is presented here, for the first time in Turkey. The use of this panel will advance knowledge of the genetic causes of infertility, enhance genetic and reproductive counseling, and eventually lead to more accurate assisted reproductive techniques.

CONCLUSION

Consequently, pre-diagnostic genes included in a custom-made NGS panel test can enhance genetic diagnostic testing and have an impact on the clinical management of male infertility. There are currently no comprehensive

systematic studies or meta-analyses on the epidemiology of male infertility, and it is unknown how common male infertility is. The need of diagnosing hereditary infertility is further supported by the epidemiological data that show infertile patients have greater morbidity and a shorter life expectancy. Finally, we demonstrated the effectiveness of NGS-based methods that additionally use pre-diagnostic genes. This gene panel may aid in determining the disorder's underlying etiology and directing clinical treatment.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors have no conflicts of interest to declare.

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