Next-generation sequencing: toward an increase in the diagnostic yield in patients with apparently idiopathic spermatogenic failure

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A large proportion of patients with idiopathic spermatogenic failure (SPGF; oligozoospermia or nonobstructive azoospermia [NOA]) do not receive a diagnosis despite an extensive diagnostic workup. Recent evidence has shown that the etiology remains undefined in up to 75% of these patients. A number of genes involved in germ-cell proliferation, spermatocyte meiotic divisions, and spermatid development have been called into play in the pathogenesis of idiopathic oligozoospermia or NOA. However, this evidence mainly comes from case reports. Therefore, this study was undertaken to identify the molecular causes of SPGF. To accomplish this, 15 genes (USP9Y, NR5A1, KLHL10, ZMYND15, PLK4, TEX15, TEX11, MEIOB, SOHLH1, HSF2, SYCP3, TAF4B, NANOS1, SYCE1, and RHOXF2) involved in idiopathic SPGF were simultaneously analyzed in a cohort of 25 patients with idiopathic oligozoospermia or NOA, accurately selected after a thorough diagnostic workup. After next-generation sequencing (NGS) analysis, we identified the presence of rare variants in the NR5A1 and TEX11 genes with a pathogenic role in 3/25 (12.0%) patients. Seventeen other different variants were identified, and among them, 13 have never been reported before. Eleven out of 17 variants were likely pathogenic and deserve functional or segregation studies. The genes most frequently mutated were MEIOB, followed by USP9Y, KLHL10, NR5A1, and SOHLH1. No alterations were found in the SYCP3, TAF4B, NANOS1, SYCE1, or RHOXF2 genes. In conclusion, NGS technology, by screening a specific custom-made panel of genes, could help increase the diagnostic rate in patients with idiopathic oligozoospermia or NOA.

INTRODUCTION

According to the World Health Organization, oligozoospermia occurs when the sperm concentration is lower than 15 × 10⁶ spermatozoa per ml or when the total sperm count is below 39 × 10⁶ spermatozoa.¹ Notably, the prevalence of oligozoospermia has increased over decades since both sperm concentration and total sperm count have decreased by half in the last 40 years.²

Despite an extensive diagnostic workup, only a minority of patients with oligozoospermia receive a specific diagnosis; thus, the etiology often remains unidentified.³ Genetic testing has been regarded as an important tool in severe male infertility diagnosis due to the high prevalence of genetic abnormalities in these patients.⁴ Currently, structural or numerical chromosomal aberrations (e.g., Klinefelter syndrome, 47,XXY karyotype), microdeletions in the azoospermia factor (AZF) region of the long arm of the Y chromosome, or mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in obstructive azoospermia are performed in clinical practice.⁵ However, data from the German Database Androbase⁶ reveal that a causal genetic diagnosis is recognized only in approximately 4% of patients with unexplained oligozoospermia and in 20% of patients with azoospermia.⁷

Human spermatogenesis is a 74-day-long complex process taking place in the seminiferous tubules by which diploid cells develop into haploid mature spermatozoa.⁸,⁹ It is thought to be orchestrated by up to 2000 genes. Among them, 600–900 genes are exclusively expressed by male germ-line cells.¹⁰–¹³

Recently, several monogenic causes of spermatogenic failure (SPGF) have been described, and overall, up to 60 candidate genes have allegedly been involved in human sperm number, motility, and/or morphological defects.¹⁴ Current evidence is mainly based on case reports or case series, although several studies that have investigated the prevalence of monogenic forms of SPGF are available.¹⁵–²⁰ However, such prevalence has mostly been explored by the research of a specific single-gene mutation among a cohort of patients with apparently idiopathic oligozoospermia, while the investigation of a broad panel of genes has not been performed so far.

Preliminary data suggest that their recognition in clinical practice would be promising. Accordingly, the analysis of testis-expressed 11 (TEX11), nuclear receptor subfamily 5, group A, member 1 (NR5A1), and doublesex- and MAB3-related transcription factor 1 (DMRT1) genes in a cohort of 80 patients with nonobstructive

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idiopathic azoospermia (NOA), no karyotype alterations, and no Yq AZF microdeletions, led to the discovery of likely pathogenic mutations in 4 patients (i.e., 5% of all cohort), raising the diagnostic rate up to 25%.7

We recently reviewed all the monogenic causes of SPGF reported so far, and we suggested that the detection of a broadened diagnostic panel of genes in patients with apparently idiopathic NOA or severe oligozoospermia may increase the probability of reaching a diagnosis.14 Therefore, the aim of the present study was to assess the prevalence of monogenic causes of SPGF through an investigation of a panel of 15 genes (Table 1) thought to regulate sperm production by next-generation sequencing (NGS) analysis in patients with idiopathic oligozoospermia or NOA.

PATIENTS AND METHODS

Patients

This was a cross-sectional study performed on patients with idiopathic oligozoospermia or NOA referred to the Division of Andrology and Endocrinology, University of Catania, Catania, Italy. Each patient underwent a comprehensive diagnostic evaluation, which included serum hormone measurement, ultrasonography (US) testicular evaluation, prostate-vesicular transrectal US, sperm, and urethral swab culture if male accessory gland infection was suspected, echo-color Doppler for varicocele detection, karyotype, Yq AZF microdeletions, and CFTR gene analysis (if needed). Sperm analysis was repeated at least twice in our Laboratory of Seminology, Division of Andrology and Endocrinology, University of Catania. Patients whose oligozoospermia or NOA was diagnosed at the end of the aforementioned diagnostic process were excluded. Instead, those whose cause of SPGF was not found were enrolled in the present study and underwent blood sampling for NGS analysis and candidate gene sequencing (Table 1).

Ethical approval

Informed written consent was obtained from each participant after full explanation of the purpose and nature of all procedures used. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki. The protocol was approved by the Internal Review Board of the Division of Andrology and Endocrinology, University of Catania.

Sperm analysis

Semen samples were collected by masturbation into a sterile container after 2–7 days of sexual abstinence and were analyzed immediately after

Table 1: Genes whose mutations cause spermatogenic failure characterized by decreased sperm number

| Gene        | Inheritance | OMIM number | OMIM phenotype | Spermatogenic defect | Mutation detection frequency | HGNC gene number | Role                      |
|-------------|-------------|-------------|----------------|----------------------|-----------------------------|------------------|--------------------------|
| NR5A1       | AR          | 184757      | SF8            | Azoospermia          | 2.2% (7/315)                | Nuclear receptor subfamily 5, group A, member 1 | Maturation arrest at spermatocyte stage16 |
| SYCP3       | AD          | 604759      | SF4            | Azoospermia          | 10.5% (2/19)                | Synaptonemal complex protein 3 | Maturation arrest17 |
| ZMYND15     | AR          | 614312      | SF14           | Azoospermia          | 1 consanguineous family     | Zinc finger, MYND-type containing 15 | Maturation arrest at spermatocyte stage16 |
| TAF4B       | AR          | 601689      | SF13           | Azoospermia          | 1 consanguineous family     | TAF4b RNA polymerase II, TATA box binding protein (TBP)-associated factor | Gonocyte proliferation16 |
| TEX11       | XL          | 300311      | SF, X-linked, 2 | Azoospermia          | 2.4% (7/289) azoospermia; 15.0% azoospermia with meiotic arrest | Testis expressed 11 | Meiotic arrest16 |
| NANOS1      | AD          | 608226      | SF12           | Azoospermia          | 2.6% (5/195)                | Nanos homolog 1 (Drosophila) | Gonocyte proliferation17 |
| PLK4        | AD          | 605031      | NA             | Azoospermia (SCOS)   | 1.2% (1/81)                 | Polo like kinase 4 | Germ-cell maintenance15 |
| MEIOB       | AR          | 617670      | SF22           | NOA                  | 1 consanguineous family     | Meiosis specific with OB domains | Maturation arrest at spermatocyte stage16 |
| SYCE1       | AR          | 611486      | SF15           | NOA                  | 1 consanguineous family     | Synaptonemal complex central element protein 1 | Meiotic arrest16 |
| USP9Y       | YL          | 400005      | SF, Y-linked, 2 | NOA                  | 3 probands (4-db DEL; DEL incl. entire gene) | Ubiquitin-specific peptidase 9, Y-linked | Its mutation is associated with hypospermatogenesis and spermatogenic arrest13 |
| SOHLH1      | NA          | 610224      | NA             | NOA                  | 2.0% (2/100)                | Spermatogenesis and oogenesis-specific basic helix-loop-helix 1 | Spermatogenesis differentiation20 |
| RHOXF2      | NA          | 300447      | NA             | Severe oligozoospermia | <1% (1/250)                | Rhox homeobox family member 2 | Its mutation is associated with hypospermatogenesis49 |
| TEX15       | AR          | 605795      | NA             | Azoospermia          | 2 families; 1 proband      | Testis expressed 15, meiosis and synopsis associated | Meiotic arrest49,10,70 |
| HSF2        | AD          | 140581      | NA             | Azoospermia          | <1% (1/766)                | Heat shock transcription factor 2 | Maturation arrest at spermatocyte stage13 |
| KLHL10      | AD          | 608778      | SF11           | OAT                  | 1.3% (7/556)               | Kelch-like family member 10 | Maturation arrest at spermatid stage14 |

AD: autosomal dominant; AR: autosomal recessive; HSF2: heat-shock transcription factor 2; KLHL10: kelch-like 10; MEIOB: meiosis-specific protein with OB domains; NA: nonobstructive azoospermia; NANOS1: nanos 2; NSI: zinc finger 1; NR5A1: nuclear receptor subfamily 5, group A, member 1; OAT: olivo-astro-enine-teratozoospermia; RHOXF2: RHox homeobox family, member 2; SF: spermatogenic failure; PLK4: polo-like kinase 4; SOHLH1: spermatogenesis- and oogenesis-specific basic helix-loop-helix protein 1; SYCE1: synaptonemal complex central element protein 1; SYCP3: synaptonemal complex protein 3; TAF4B: RNA polymerase II, TATA box-binding protein-associated factor; TEX11: testis-expressed 11; TEX15: testis-expressed 15; ZMYND15: zinc finger mynd-containing protein 15; USP9Y: ubiquitin-specific peptidase 9, Y chromosome; NA: not available; XL: X-linked; YL: Y-linked
liquefaction. According to the 2010 WHO guidelines, each sample was evaluated for seminal volume, pH, sperm count, progressive motility, morphology, and round cell concentration. Bioinformatic and genetic analysis

Genetic analysis was performed using an NGS approach and a custom-made gene panel designed to include known genes involved in SPGF (Table 1). A MiSeq personal sequencer (Illumina, San Diego, CA, USA) was used for NGS. Sanger sequencing was performed for this gene panel when the target region coverage was less than 10 reads and for the family segregation study. All laboratory methods have been described elsewhere. Briefly, 50 ng of genomic DNA was fragmented by enzymatic methods (Nextera Transposome System, Thermo Scientific, Waltham, MA, USA), and the target regions were enriched by Illumina Nextera Rapid Capture Enrichment (Thermo Scientific). Sequencing was performed by Illumina MiSeq using a paired-end protocol and 150 bp long reads. Nucleotide alterations were analyzed and validated by PCR. Fastq (forward-reverse) files were obtained after sequencing. Read alignment was performed using BWA (0.7.17-r1188) software (GitHub, San Francisco, CA, USA). Duplicates were removed using the SAMBAMBA (0.6.7) program (GitHub), and GATK (4.0.0; GitHub) was used for realignment. We searched the international databases dbSNP (www.ncbi.nlm.nih.gov/SNP/) and Human Gene Mutation Database professional (HGMD; http://www.biobase-international.com/product/hgmd) for all nucleotide changes. In silico evaluation of the pathogenicity of nucleotide changes in exons was performed using the Variant Effect Predictor tool (http://www.ensembl.org/Tools/VEP) and MutationTaster (http://www.mutationtaster.org). Minor allele frequencies (MAFs) were checked in the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/). All variants were evaluated according to the American College of Medical Genetics and Genomics guidelines. Nucleotide variations next to the 3’ UTR are indicated with **. Results

Overall, 25 unrelated patients satisfied the inclusion criteria and showed idiopathic oligozoospermia (n = 18) or NOA (n = 7). At enrollment, their clinical features, including age, hormone serum levels, and testicular volume, are reported in Table 2.

We found a total of 16 different rare nucleotide alterations, 12 of which have never been associated with a pathological phenotype. Rare variants with a known pathogenic effect in the NR3A1 (c.1063G>A p.Val355Met and c.1052C>T p.Ala351Val) and TEX11 (c.2288T>C p.Val763Ala) genes were identified in 3/25 (12.0%) patients with idiopathic oligozoospermia and one with NOA. Rare mutations have been reported in patients with apparently idiopathic oligozoospermia and one with NOA. In particular, a patient with NOA, increased gonadotropin levels, and low testicular volume had both the c.634G>A p.Asp212Asn and the c.4G>A variants (Table 4). The USP9Y c.3178G>A p.Ala1060Thr variation was observed in 2 patients with oligozoospermia. Similarly, the same KLHL10 rare variation (c.5G>G) was identified in one patient with oligozoospermia and one with NOA. Rare nucleotide variations in SOHLH1 were identified in two different patients. The remaining variants were found in one patient each.

Discussion

Disorders of spermatogenesis are widespread throughout the world. In particular, the prevalence of oligozoospermia has increased in the last four decades, as meta-regression data suggest. Worriedly, despite a comprehensive diagnostic workup, only a minority of patients receive a definite diagnosis, indicating the need to revise and implement the diagnostic tools currently adopted in clinical practice.

A genetic cause of oligozoospermia or NOA is thought to occur in the most severe cases. Although an increasing number of gene mutations have been reported in patients with apparently idiopathic oligozoospermia or NOA, only screening for chromosomal abnormalities, Yq microdeletions, or CFTR mutations is routinely performed. Currently, NGS technology can be successfully used to perform molecular screening of a wide custom-made gene panel in a large cohort of patients with reasonable effort in terms of cost, workload, and time.

In the present study, we analyzed a panel of 15 genes known to be involved in spermatogonia proliferation, spermatocyte meiotic divisions, and spermatid maturation whose loss-of-function mutations have been reported to play a role in SPGF (Table 1). We studied 25 patients, 18 with idiopathic oligozoospermia and 7 with idiopathic NOA, carefully selected after a complete diagnostic workup. Overall, we found 17 rare genetic variants, 13 of which have never been reported before.

We identified three variants with a clearly known pathogenic effect in three patients (12.0%). Indeed, these variants have already been reported, and their causal role has been proven by functional studies. NR5A1 is a gene with an autosomal dominant inheritance, encoding a nuclear receptor transcription factor involved in adrenal and gonadal development, steroidogenesis, and reproduction. Mutations in this gene have been described in patients with oligozoospermia or NOA. The NR5A1 Val355Met mutation has been reported to interfere with protein function. Indeed, Val355 is a conserved amino acid residue belonging to a functional domain of the protein. The Val355Met variant

Table 2: Demographic and clinical characteristics of the patients enrolled in this study

|                      | Oligozoospermic patients (n=18) | Azoospermic patients (n=7) |
|----------------------|--------------------------------|---------------------------|
| Age (year)           | 40.3±10.3                      | 35.3±9.2                  |
| FSH (mU/ml i-1)      | 6.6±3.7                        | 15.4±12.1                 |
| LH (mU/ml i-1)       | 4.2±2.1                        | 7.4±5.8                   |
| Total testosterone (ng ml-1) | 4.9±1.6               | 4.7±1.2                   |
| Right testicular volumee (ml) | 10.5±3.4            | 13.7±7.6                  |
| Left testicular volume (ml) | 11.3±3.5              | 13.4±8.2                  |
| Sperm concentration (×10⁶ per ejaculate) | 8.8±11.8          | 0                        |
| Total sperm count (×10⁶ per ejaculate) | 26.6±43.1           | 0                        |

*Testicular volumes were evaluated by ultrasound. The results are presented as the mean±standard deviation.

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halves the protein activity in several different assay systems, resulting in a heterozygous partial NR5A1 loss of function. Similarly, Ala351 is a conserved amino acid residue of a functional domain of the NR5A1 protein. Ala351Val has already been reported in a case of a disorder of sexual development. Furthermore, a variant of the same codon Ala351Glu has been reported in XY sex reversal.

**Table 3: Variants found in the patients with idiopathic oligozoospermia enrolled in this study**

| Patient | Gene/isofrom (inheritance) | Variant (zygosity) | Variant ID | GnomAD minor allele frequency (%) | Polyphen-2 | SIFT | MutationTaster | CADD score | Pathogenicity confirmed by functional studies | Interpretation |
|---------|----------------------------|-------------------|-----------|----------------------------------|------------|------|----------------|----------|----------------------------------------|----------------|
| Patient 2 | USP9YI NM_004654 (YL) | c.3178G>A; p.Ala1060Thr (hem) | rs20320 | 0.8 | Benign | Tolerated / | 17.34 | No | Likely benign |
| Patient 4 | NRS5A1/ NM_004654 (YL) | c.1063G>A; p.Val355Met (het) | rs371701248 | 0.01 | Possibly damaging | Deleterious | Disease causing | 26.0 | Yes* | Pathogenic |
| Patient 5 | KLHL10I/ NM_152467 (AD) | c.5C>G (het) | rs189686821 | 0.5 | / | / | Disease causing | 19.92 | No | Uncertain |
| Patient 6 | ZMYND15/ NM_001136046 (AD) | c.2015G>A; p.Arg672His (het) | rs148900412 | 0.2 | Probably damaging | Disease causing | Disease causing | 28.4 | No | Uncertain |
| Patient 7 | MEIOB/ NM_152467 (AD) | c.318C>A; p.Ser106Arg (het) | rs143362679 | 0.1 | Benign | Tolerated | Disease causing | 18.28 | No | Uncertain |
| Patient 8 | PLK4/ NM_014264 (AD) | c.170>A; p.Gly56Glu (het) | rs149003893 | 0.1 | Probably damaging | Deleterious | Disease causing | 28.3 | No | Uncertain |
| Patient 10 | NRS5A1/ NM_004959 (AD) | c.1052C>T; p.Ala351Val (hem) | rs759071081 | 0.005 | Probably damaging | Deleterious | Disease causing | 26.3 | Yes† | Pathogenic |
| Patient 11 | TEX1/ NM_031271 (AR) | c.7118G>A; p.Ser2373Asn (het) | rs757169474 | 0.0008 | Benign | Tolerated | Polymorphism | 0.643 | No | Likely benign |
| Patient 12 | SOHLH1/ NM_00102415 (AD) | c.916C>A; p.Leu306Met (het) | rs144035874 | 0.4 | Possibly damaging | Disease causing | Polymorphism | 20.4 | No | Uncertain |
| Patient 13 | MEIOB/ NM_152764 (AR) | c.634G>A; p.Asp212Asn (het) | rs62617828 | 0.8 | Possibly damaging | Tolerated | Disease causing | 24.8 | No | Uncertain |
| Patient 14 | TEX1/ NM_001003811 (XL) | c.228T>C; p.Val763Ala (hem) | rs200139216 | 0.02 | Possibly damaging | Tolerated | Polymorphism | 19.46 | Yes‡ | Pathogenic |

*Philbert et al. 2007; †Philbert et al. 2011; Rocca et al. 2018; ‡Bouilly et al. 2016; Zhao et al. 2014; §Yang et al. 2015. AD: autosomal dominant; AR: autosomal recessive; het: heterozygous; hem: hemizygous; XL: X-linked; YL: Y-linked; CADD: combined annotation-dependent depletion; SIFT: sorting intolerant from tolerant; USP9Y: ubiquitin-specific protease 9, Y chromosome; NRS5A1: nuclear receptor subfamily 5, group A, member 1; KLHL10: kelch-like 10; MEIOB: meiosis-specific protein with OB domains; SOHLH1: spermatogenesis- and oogenesis-specific basic Helix-Loop-Helix protein 1; ZMYND15: zinc finger mynd-containing protein 15; PLK4: polo-like kinase 4; TEX1: testis-expressed 15; TEX12: testis-expressed 11; /: no test can be performed

**Table 4: Variants found in the patients with idiopathic nonobstructive azoospermia enrolled in this study**

| Patient | Gene/isofrom (inheritance) | Variant (zygosity) | Variant ID | GnomAD minor allele frequency (%) | Polyphen-2 | SIFT | MutationTaster | CADD score | Pathogenicity confirmed by functional studies | Interpretation |
|---------|----------------------------|-------------------|-----------|----------------------------------|------------|------|----------------|----------|----------------------------------------|----------------|
| Patient 2 | KLHL10I/ NM_152467 (AD) | c.5C>G (het) | rs189686821 | 0.5 | / | / | Disease causing | 19.92 | No | Uncertain |
| Patient 4 | SOHLH1/ NM_001012415 (AD) | c.866G>C; p.Glu290Gln (het) | rs145861345 | 0.03 | Benign | Tolerated | Polymorphism | 9.066 | No | Uncertain |
| Patient 5 | MEIOB/ NM_152764 (AR) | c.4G>A (het) | rs369643368 | 0.005 | / | / | Polymorphism | 0.396 | No | Uncertain |

AD: autosomal dominant; AR: autosomal recessive; het: heterozygous; XL: X-linked; YL: Y-linked; CADD: combined annotation-dependent depletion; SIFT: sorting intolerant from tolerant; KLHL10: kelch-like 10; MEIOB: meiosis-specific protein with OB domains; SOHLH1: spermatogenesis- and oogenesis-specific basic Helix-Loop-Helix protein 1; /: no test can be performed

TEX11, mapping to the Xq13.1 chromosome, is a meiosis-specific factor that plays a role in double-strand DNA breaks (DSB) repair. Mutations in this gene have already been reported in patients with spermatogonial arrest at the meiotic phase. The Val673Ala variant has been reported in NOA patients, and functional studies have shown its pathogenic role.
Among the newly reported variants, 11 may be disease-causing. A genetic variant was considered to be likely disease causing if it had a very low frequency in the general population (gnomAD minor allele frequency, detailed in Table 3 and 4), it modified a conserved amino acid residue, and was predicted to significantly affect the protein structure or function by in silico evaluation. Functional or segregation studies are warranted to confirm their pathogenic role. By contrast, pathogenic variants were defined in the case of existing functional studies documenting their disease-causing role.

USP9Y, mapping to the Yq11.221 chromosome, encodes ubiquitin-specific protease 9 and maps inside the so-called AZFa region. Mutations and deletions in this gene have been reported in NOA patients, representing the Y-linked SPGF phenotype (OMIM 415000). However, a 513 594 bp deletion in the AZFa region encompassing the USP9Y gene has been described in a normozoospermic man and his brother and father. Thus, the role of this gene in SPGF has yet to be clarified. Interestingly, we found the Ala1060Thr variation, which has never been reported, in two patients with oligozoospermia. Ala1060 is a conserved amino acid residue whose variation may potentially impact protein function. However, functional or segregation studies are needed to clarify its role.

KLHL10, with an autosomal dominant inheritance, is the hallmark of SPGF10 (OMIM 608778). It encodes an evolutionarily conserved protein specifically expressed in spermatids. The c.674A>C p.Gln21Pro and the c.937G>A p.Ala313Thr variants affecting protein homodimerization have been reported in patients with oligozoospermia. We also found a novel variant in the 3’ UTR region, the c.5C>G variant, in one patient with oligozoospermia and one with NOA. The functional role of this rare nucleotide alteration should be investigated.

MEIOB encodes an evolutionarily conserved protein in vertebrates with single-stranded DNA (ssDNA) binding sites. It has been implicated in the pathogenesis of SPGF only recently. The mouse orthologue protein is indispensable for crossing-over, and meiobdeficient mice show SPGF due to meiotic arrest. Accordingly, few MEIOB homozygous mutations have been reported in patients with NOA due to spermatocyte meiotic arrest. Overall, we identified four novel variants (c.318C>A p.Ser106Arg, c.634G>A p.Asp212Asn, c.643T>G p.Ser215Ala and c.4G>A), two of them (c.634G>A p.Asp212Asn and c.4G>A) occurring twice in our cohort. In particular, c.634G>A p.Asp212Asn is a missense variant affecting a conserved amino acid residue without altering its chemical properties. c.4G>A maps to the 3’ UTR region of the gene. Notably, these variants have both been found in a patient with NOA, increased gonadotropin levels and low testicular volume, all signs of impaired testicular function.

PLK4 is involved in germ-cell maintenance. In humans, heterozygous variations have been reported in NOA patients. We reported two novel variants (c.17G>A p.Gly6Glu and c.1556G>C p.Trp519Ser) in a patient with oligozoospermia, both affecting conserved amino acid residues and were predicted to significantly affect the protein structure or function. The c.868G>C p.Glu290Gln nucleotide alteration was found in a patient with NOA. It has never been reported so far, and it involves a conserved amino acid residue, with no consequences on the amino acid nature. Its functional role should be investigated.

CONCLUSION

The results of this pilot study contribute to further expanding the current knowledge on nucleotide alterations of SPGF-related genes. As pathogenic variants, they were found in 12.0% of the enrolled patients; hence, this panel (Table 1) should be used to assess the prevalence of disease-causing genetic variants in a wider sample size prior to its introduction in the screening of these mutations in clinical practice. The investigation of this wide custom-made gene panel in patients with idiopathic oligozoospermia or NOA by NGS technology may reasonably increase the rate of diagnosis. Furthermore, likely pathogenic variants need to be assessed in fertile patients to ascertain their role in the etiology of the disease and to be further investigated by functional or segregation studies. Finally, the possible prognostic role in the evaluation of testicular sperm retrieval rate, pregnancy outcome, and health risk for the offspring needs to be addressed in the future.

AUTHOR CONTRIBUTIONS

RC conceived the study, participated in data analysis, and wrote the original draft. RAC participated in data analysis and in project supervision. SP performed the genomic studies and participated in the writing of the original draft. FB participated in the writing of the original draft. GG participated in the genomic studies and performed the statistical analysis. MB participated in the draft of the manuscript and in project supervision. SLV participated in project supervision and in review and editing of the final version of the manuscript. AEC conceived the study, supervised the project, and edited the final version of the manuscript. All authors have read and approved the final version of the manuscript. All authors declared no competing interests.

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