New perspectives in the genetic diagnosis of male infertility

The current issue of the *Croatian Medical Journal* features two interesting articles on reproductive health. Sengun et al (1) present their findings of novel mutations in the gene coding for FK506 binding protein-like (*FKBPL*) associated with male infertility, while Bilić et al (2) discuss the benefits of ovarian tissue cryopreservation. In spite of tremendous advances in the field, various aspects of reproductive health, particularly infertility, still necessitate further study and development of novel diagnostic and therapeutic approaches.

Infertility, defined by the World Health Organization as the failure to achieve conception after 12-24 months of unprotected sexual intercourse (3), affects about one out of six couples of childbearing age in Western countries (4). Male factor infertility is identified in about half of the cases (5) and most of the times presents as spermatogenesis failure (SPGF). Spermatogenesis is a 74-day-long process involving up to 2000 genes. Among them, 600 to 900 are exclusively expressed by the male germ-line (6-8). Despite a comprehensive diagnostic workup, in the majority of cases the etiology of SPGF remains elusive. A monocentric study on 1737 infertile patients reported idiopathic oligozoospermia in ~75% of cases (9). Classic genetic screening can identify the cause of male infertility in only 5% of unselected patients and in 20% of patients with non-obstructive azoospermia (NOA) (10). In the remaining cases, the etiology of infertility is elusive.

In recent years, several mutations of genes involved in different steps of spermatogenesis, such as spermatogonia proliferation or meiotic division of primary spermatocytes, have been reported capable of interfering with murine spermatogenesis (11). Interestingly, many of them have been validated in infertile patients, as shown in case reports or case series. Thus, the inclusion of a comprehensive gene panel in the diagnostic algorithm of male infertility could raise the diagnostic yields (11). The first study addressing this issue analyzed a panel of three genes: *NR5A1*, *DMRT1*, and *TEX11*, in a cohort of 80 patients with NOA. *NR5A1* (9q33.3) encodes for a transcription factor involved in the regulation of genes playing a role in steroidogenesis. Its mutation has been associated with a wide spectrum of phenotypes, ranging from 46,XY partial and complete gonadal dysgenesis, to hypospadias, micropenis, anorchia, and, in otherwise healthy men, oligozoospermia or NOA. *DMRT1* (9p24.3) encodes for a testis-specific transcription factor acting in testis differentiation, whose mutations have been reported in patients with NOA. Finally, *TEX11* (Xq13.1) encodes for a meiosis-specific factor, involved in double-strand breaks DNA repair, which may play a role in NOA pathogenesis due to meiotic arrest (11). Out of 80 patients with NOA investigated for *NR5A1*, *DMRT1*, and *TEX11* gene mutations, the authors reported pathogenic variants in four, thus raising the diagnostic yield by 25% (10). A subsequent study has developed a gene panel including 15 genes (Table 1) involved in germ-cell proliferation and meiotic division. Interestingly, pathogenic mutations of *NR5A1* and *TEX11* genes were reported in 3/25 patients, increasing the diagnostic rate by 12%. Noteworthy, 11 likely pathogenic variants meriting functional analysis or segregation studies were also observed.
These data highlight the importance of designing a comprehensive and accurate gene panel to be used in patients with otherwise unexplained SPGF. Furthermore, next-generation sequencing makes this analysis widely accessible from both an economic and a geographic point of view. An appropriate gene panel for SPGF could facilitate the identification of the genetic cause of infertility (when present), but in the future it may also represent a diagnostic test predicting sperm recovery after testicular sperm extraction (TESE). Accordingly, a study matching the results of testicular histology with those of genetic testing has been published and others are currently ongoing. Interestingly, mutations (eg, deletions, missense, stop-gain) of specific genes involved in meiosis (eg, M1AP, ADAD2, TERB1, SHOC1, MSH4, RAD21L1, TEX14, DMRT1, TEX1, SYCE1, MEIOB, MEI1, STAG3-a) have been reported in patients with meiotic arrest (13,14).

If validated, these gene variations may be included in a pre-TESE prognostic gene panel, which may help to determine the chance of sperm recovery. Finally, another challenge in this field is to understand the implications of the transmission of these gene mutations to the offspring.

In conclusion, the evidence favoring the inclusion of SPGF monogenic mutation assessment in the diagnostic workup of male infertility is accumulating. Despite this, a comprehensive panel has not yet been validated, although some gene mutations are more frequently present than others (eg, NR5A1 or TEX11), and others have recently been discovered. Since more than 2000 genes are involved in spermatogenesis, we are still far from a comprehensive view of the monogenic etiology of SPGF. However, in the near future, this evidence is likely to practically affect the diagnostic workup and decision-making algorithms of male infertility.

**TABLE 1.** Genes whose mutations cause spermatogenic failure characterized by a decreased sperm number (12)*

| Gene   | Inheritance | OMIM number | OMIM phenotype | Spermatogenic defect                  | Mutation detection frequency | HGNC gene number |
|--------|-------------|-------------|----------------|---------------------------------------|-----------------------------|------------------|
| NR5A1  | AR          | 184757      | SF8            | Azoospermia                           | 2% (7/315)                  | Nuclear receptor subfamily S, group A, member 1 |
| SYCP3  | AD          | 604759      | SF4            | Azoospermia                           | 10.5% (2/19)                | Synaptonemal complex protein 3 |
| ZMYND15| AR          | 614312      | SF4            | Azoospermia                           | 1 consanguineous family     |  |
|        |             |             |                | Synaptonemal complex protein 3        | 10% (2/20)                  |  |
| TAF4B  | AR          | 601689      | SF13           | Azoospermia                           | 1 consanguineous family     |  |
|        |             |             |                | Synaptonemal complex protein 3        | 10% (2/20)                  |  |
| TEX11  | XLR         | 300311      | SF, X-linked   | Azoospermia                           | 1% (1/42)                   |  |
|        |             |             | 2              | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| NANOS1 | AD          | 608226      | SF12           | Azoospermia                           | 1% (1/42)                   |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| PLK4   | AD          | 605031      | -              | Azoospermia                           | 1% (1/42)                   |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| MEIOB  | AR          | 617670      | SF22           | NOA                                   | 1 consanguineous family     |  |
|        |             |             |                | Meiosis specific with OB domains      | 1% (1/42)                   |  |
| SYCE1  | AR          | 611486      | SF15           | NOA                                   | 1 consanguineous family     |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| USP9Y  | YL          | 400005      | SF, Y-linked   | NOA                                   | 3 probands (4-db DEL; DEL incl. entire gene) |  |
|        |             |             | 2              | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| SOHLH1 | -           | 610224      | -              | NOA                                   | 2% (2/100)                  |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| RHOXF2 | -           | 300447      | -              | Severe oligozoospermia                | <1% (1/250)                 |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| TEX15  | AR          | 605795      | -              | Azoospermia                           | 2 family; 1 proband        |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| HSF2   | AD          | 140581      | -              | Azoospermia                           | 2 family; 1 proband        |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |
| KXHL10 | AD          | 608778      | SF11           | OAT                                   | 2 family; 1 proband        |  |
|        |             |             |                | Synaptonemal complex protein 3        | 1% (1/42)                   |  |

*Abbreviations: OMIM – Online Mendelian Inheritance in Man; HGNC – Hugo Gene Nomenclature Committee; AD – autosomal dominant; AR – autosomal recessive; NOA – non-obstructive azoospermia; OAT – oligo-astheno-teratozoospermia; SF – spermatogenic failure.
References
1. Sengun DA, Tanoglu EG, Ulucan H. A novel mutation in FK506 binding protein-like (FKbPL) causes male infertility. Croat Med J. 2021;62:227-32.
2. Bilić K, Vilaj M, Golubić-Ćepulić B, Ježek D. Ovarian tissue bank. Croat Med J. 2021;62:297-9.
3. World Health Organization. Report of the meeting on the prevention of infertility at the primary health care levels. Geneva: WHO; 1983.
4. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37. Medline:25928197 doi:10.1186/s12958-015-0032-1
5. Valenti D, La Vignera S, Condorelli RA, Rago R, Barone N, Vicari E, et al. Follicle-stimulating hormone treatment in normogonadotropic infertile men. Nat Rev Urol. 2013;10:55-62. Medline:23229508 doi:10.1038/nrurol.2012.234
6. Schultz N, Hamra FK, Garbers DL. A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. Proc Natl Acad Sci U S A. 2003;100:12201-6. Medline:14526100 doi:10.1073/pnas.1635054100
7. Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med. 2008;14:1197-213. Medline:18989107 doi:10.1038/nm.1895
8. Yan W. Male infertility caused by spermiogenic defects: lessons from gene knockouts. Mol Cell Endocrinol. 2009;306:24-32. Medline:19481682 doi:10.1016/j.mce.2009.03.003
9. Punab M, Poolamets O, Paju P, Vihljaie V, Pomm K, Ladva R, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod. 2017;32:18-31. Medline:27864361 doi:10.1093/humrep/dey142
10. Tüttelmann F, Ruckert C, Röpke A. Disorders of spermatogenesis: Perspectives for novel genetic diagnostics after 20 years of unchanged routine. Med Genetik. 2018;30:12-20. Medline:29527098 doi:10.1007/s11825-018-0181-7
11. Cannarella R, Condorelli RA, Duca F, La Vignera S, Calogero AE. New insights into the genetics of spermatogenic failure: a review of the literature. Hum Genet. 2019;138:125-40. Medline:30656449 doi:10.1007/s00439-019-01974-1
12. Cannarella R, Condorelli RA, Paolacci S, Barbagallo F, Guerri G, Bertelli M, et al. Next-generation sequencing: toward an increase in the diagnostic yield in patients with apparently idiopathic spermatogenic failure. Asian J Androl. 2021;23:24-9. Medline:32655042 doi:10.4103/aja.aja_25_20
13. Wyrwoll MJ, Temel ŞG, Nagirnaja L, Oud MS, Lopes AM, van der Heijden GW, et al. Bi-allelic mutations in M1AP are a frequent cause of meiotic arrest and severely impaired spermatogenesis leading to male infertility. Am J Hum Genet. 2020;107:342-51. Medline:32673564 doi:10.1016/j.ajhg.2020.06.010
14. Krausz C, Riera-Escamilla A, Moreno-Mendoza D, Holleman K, Cioppì F, Algaba F, et al. Genetic dissection of spermatogenic arrest through exome analysis: clinical implications for the management of azoospermic men. Genet Med. 2020;22:1956-66. Medline:32741963 doi:10.1038/s41436-020-0907-1