Abstract citation ID: deac107.022

P-024 Identification of spermatogenic infertility phenotypes using next generation sequencing

G.. Eva M[1], F.M. Lozano[1], B. Lledo[1], A. Turienzo[1], A. Cascales[1], J.A. Ortiz[1], R. Morales[1], A. Fuentes[2], A. Bernabeu[2], R. Bernabeu[2]

[1]*Instituto Bernabeu, IB Biotech, Alicante, Spain*
[2]*Instituto Bernabeu, Reproductive Medicine, Alicante, Spain*

Study question: Can next generation sequencing (NGS) contribute to diagnoses male idiopathic infertility?

Summary answer: A male factor gene panel identifies pathogenic variants associated to spermatogenic failure in oligozoospermia and cryptozoospermia patients.

What is known already: In 50% of cases, infertility is due to a male factor problem. Although the causes of male infertility are heterogeneous, genetic causes account for approximately 30% of cases. Some phenotypes have been associated with specific genetic disorders such as chromosomal abnormalities and chromosome Y microdeletions. However, current genetic studies explain only 4% of cases, whilst most cases of male factor infertility remain without a clear diagnosis. Therefore, new techniques that explain the cause of male infertility are needed. Advances in NGS allowed us to study a large number of genes involved in spermatogenesis process in patients with idiopathic infertility.

Study design, size, duration: A retrospective study was performed from July 2020 until May 2021. A total of 30 patients with abnormal seminal count parameters (oligozoospermic and cryptozoospermic) were included in the male factor gene panel study. Patients carrying Y-chromosome microdeletions or abnormal karyotype were excluded. The control group included 20 normozoospermic healthy donors selected on the basis of normal semen parameters according to the WHO criteria (2010).

Participants/materials, setting, methods: Genomic DNA extraction from blood-EDTA of the patients was performed using the commercial MagMax DNA MultiSample Ultra kit and the King-Fisher automated extractor (ThermoFisher®). Next Generation Sequencing (NGS) was done using a panel with 426 genes involved in the spermatogenesis process. Panel sequencing for identification of genetic variants was performed using Nextera Enrichment technology (Illumina®). FASTAQ data were processed using BWA and GATK algorithms. VCF files were analyzed using Variant Interpreter software.

Main results and the role of chance: After data analysis, we observed that eight of the thirty patients studied were carriers of mutations in least one of the genes included in the panel (8/30, 26.7%). We identified the following pathogenic variants: a missense mutation (Phe1052Val) and a deletion (Phe508del) of *CFTR* gene (2/30, 6.6%), two frameshifts (Asp128GlufsTer34 and Lys1299Ter) of *CEP290* (2/30, 6.6%), a missense mutation (Tyr284Cys) of *GNRHR* gene (1/30, 3.3%), a missense mutation (Tyr416Cys) of *SCN5A* gene (1/30, 3.3%), a deletion (Ser83del) of *NANOS1* gene (1/30, 3.3%), a stop gained in splice region Arg341Ter of *TEX14* gene (1/30, 3.3%), a splicing donor c.362 + 2T>C of *ESR2* gene (1/30, 3.3%) and a missense mutation (Ser321Leu) of *DNAH5* gene (1/30, 3.3%), which are related to spermatogenesis failure. Additionally, some variants classified as benign have been identified, which are not associated with pathogenicity. All the variants identified are related with male infertility, affecting spermatogenesis process, such as congenital bilateral absence of the vas deferens (*CFTR*), reproductive system syndrome (*CEP190*), endocrine disorder (*GNRHR*, hypogonadotropic hypogonadism), testis expressed (*SCN5A*), spermatogenic failure (*NANOS1*, *TEX14* and *ESR2*) and syndromic infertility (*DNAH5*). Nevertheless, no pathogenic mutations associated to spermatogenic failure were observed in the control group.

Limitations, reasons for caution: The main limitation of this study is the small number of patients included. Further studies including a higher number of males with idiopathic infertility are warranted to confidently link the genetic variants included in our gene panel to spermatogenic failure.

Wider implications of the findings: The gene list included in our panel represents a step-forward in the diagnosis screening of males with altered sperm parameters. Our results may add in the knowledge of male factor infertility in order to provide etiologic factors towards a personalized treatment and adequate genetic counselling.

Trial registration number: Not applicable