ISSN 2304-3415, Russian Open Medical Journal
2022. Volume 11. Issue 1 (March). Article CID e0101
DOI: 10.15275/rusomj.2022.0101

Review

Overview of current NGS testing for male factor infertility

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Received 24 July 2021, Revised 8 November 2021, Accepted 1 February 2022

Abstract: *Aim* — Infertility is a global health problem. The next-generation sequencing and panel testing are offering new opportunities to further diagnose the reason for male infertility. The aim of this paper is to provide a better insight into the currently available panels for male infertility due to impaired spermatogenesis.
Methods — We conducted research in the Genetic testing registry by using the keywords „infertility", „male infertility". We also gathered information about the number of tested genes, coverage of the panels, turnaround time, and any additional tests, which could be ordered.
Results — As a result there were eleven laboratories, offering panel testing for male infertility, which tested for 230 different genes, but 65 genes (28.26%) from the different panels had an uncertain role for the tested condition. Cystic fibrosis transmembrane conductance regulator was the only gene, suggested by all laboratories.
Conclusions — Next-generation sequencing could be extremely helpful in the diagnostic process of male infertility. However, clinicians should be aware that some of the included genes have an uncertain role for male infertility.

Keywords: male infertility, next-generation sequencing, panel testing.

Cite as Levkova M, Chervenkov T, Hachmeriyan M, Angelova L. Overview of current NGS testing for male factor infertility. *Russian Open Medical Journal* 2022; 11: e0101.

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Introduction

Infertility is a global health issue, which affects men and women in equal proportions [1]. The male infertility could be due to endocrine disorders, cryptorchidism, varicocele, testicular trauma, environmental factors, etc. [2]. Also, in about 15% of the cases, there is a genetic reason for the absent male fertility, with most common causes Klinefelter syndrome, Y microdeletions, chromosomal rearrangements, mutations in the *CFTR* (Cystic fibrosis transmembrane conductance regulator) gene, Kalman syndrome, and some others [3].

In the last couple of years, male infertility has gained more importance due to the advancing genetic technologies such as array-comparative genomic hybridization (CHG) and next-generation sequencing (NGS), which led to the discovery of new candidate genes [3, 4]. NGS includes targeted gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS). The whole genome sequencing is used to search for variants in the full human DNA sequence, including non-coding and inter gene regions. Whole exome sequencing includes testing of all coding regions, but non-coding regions such as regulatory regions are excluded from this type of genetic analysis. Panel gene testing refers to offering a specific set of genes, which are chosen based on previously published data about association between these genes and a specific disorder. The number of the included genes could vary [5].

Despite the advancement in the genetic etiology of male infertility, the genetic testing has not changed much. Karyotyping together with analysis for Y microdeletions remain the main tests, which are offered to infertile male patients. This could explain the fact that in around 50% of the cases male infertility remains unexplained and is described as idiopathic [2]. In order to provide better care for these patients, the clinicians should consider offering them testing for new genetic variants as a part of a multigene NGS panel testing. These panels provide molecular analysis for a great variety of genes, associated with male infertility and are cost-effective.

Nevertheless, choosing the right panel could be very challenging. The aim of this paper is to provide a better insight into the available on the market panels for male infertility, their coverage, turnaround time and to help clinicians provide better care to their patients.

Material and Methods

In order to estimate what was the current status of genetic testing for male infertility due to impaired spermatogenesis and what NGS panels were offered on the market, we conducted a search in the Genetic testing registry (GTR), brought by National Center for Biotechnology Information (NCBI). We used the keywords „infertility", „male infertility" and reviewed the available tests.

Table 1 List of Genetic laboratories, offering NGS panel testing for male infertility. Coverage for Laboratory VII was not available

Name of the laboratory	*Total genes tested in a panel*	*Percentage of genes with uncertain significance*	*Sensitivity of the panel*	*Turn -around time (days)*	*Testing for sex aneuploidy available*	*Testing for Ymicrodeletions available*
Laboratory I	45	4.45%	96% at 20x	21-35	No	No
Laboratory II	20	27.78%	≥99% at 20x	21-28	Yes, by CNV	Yes, included in the panel
Laboratory III	5	20%	~99% at 20x	21	Yes, by cytogenetic analysis	Yes, included in the panel
Laboratory IV	107	9.35%	≥ 98% at 20x	18	Yes, by CNV	No
Laboratory V	94	15.62%	≥99.5% at 20x	25	Yes, by CNV and by MLPA for aneuploidy	Additionally available
Laboratory VI	116	8.62%	>95% at >100x	60	Yes, by CNV	Additionally available
	21	0%	>95% at >100x	45	No	Additionally available
Laboratory VII	29	0%	NA	56	Yes, by CNV	Additionally available
Laboratory VIII	138	29.71%	99%	42-63	Yes, by CNV	Additionally available
Laboratory IX	Up to 62	12.77%	99,8% of the target region at a minimum of 30x.	28-42	Yes, by CNV	Additionally available
Laboratory X	12	8.33%	98% at 20x	30	Yes, by CNV	Additionally available
Laboratory XI	5	20.0%	~99%	28	Yes, by cytogenetic analysis	Yes, included in the panel

Table 2 Genetic syndromes and a list of genes with uncertain significance, included in the panels of all eleven laboratories. The genetic conditions and the genes are listed alphabetically

Disorders with main symptom male infertility	
17-alpha-hydroxylase/17,20-lyase deficiency; 46 XY Gonadal Dysgenesis and Gonadal Dysgenesis; 46 XY partial gonadal dysgenesis, with minifascicular neuropathy; 46 XY sex reversal; Adrenal hyperplasia, congenital, due to 11-beta-hydroxylase deficiency; Adrenal hyperplasia, congenital, due to 3-beta-hydroxysteroid dehydrogenase 2 deficiency; Adrenal insufficiency, congenital, with 46 XY sex reversal, partial or complete; Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis; Aromatase deficiency; Autoimmune polyendocrinopathy syndrome; Borjeson-Forssman-Lehmann syndrome; Campomelic dysplasia with autosomal sex reversal; Ciliary dyskinesia; Complete androgen insensitivity syndrome;	Congenital bilateral absence of vas deferens; Cryptorchidism; Cystic fibrosis; Deafness-Infertility Syndrome; Early onset obesity with hypogonadotrophic hypogonadism; Hypogonadotropic hypogonadism with or without anosmia; Hypospadias; Kallmann Syndrome; Leydig cell hypoplasia with hypergonadotropic hypogonadism; Palmoplantar hyperkeratosis and true hermaphroditism; Panhypopituitarism; Persistent Mullerian duct syndrome; Pituitary hormone deficiency, combined; Pseudohermaphroditism, male, with gynecomastia; Pseudovaginal perineoscrotal hypospadias; Spermatogenic failure; Testicular anomalies with or without congenital heart disease; X-linked congenital adrenal hypoplasia and hypogonadotropic hypogonadism;
Disorders with minor symptom male infertility	
Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency; Alpha-thalassemia/mental retardation syndrome; Alstrom syndrome; Bardet-Biedl syndrome Denys-Drash syndrome; Hydranencephaly with abnormal genitalia;	Joubert syndrome; Mental Retardation, Truncal Obesity, Retinal Dystrophy, And Micropenis Syndrome; Mulibrey Nanism; Nephronophthisis; Retinitis pigmentosa; Senior-Loken syndrome; STAR syndrome;

Genes with uncertain role for male infertility								
BMP4	FGFR2	HFE	MAGEB4	NR2E3	PROM1	SLC5A5	TG	TSHR
BNC2	FOXE1	IFT172	MYO7A	PANK2	PRPH2	SLC9A3	THRA	TTF1
CASR	FOXL2	IGSF1	NKX2-1	PAX8	RDH5	SOX10	THRB	USP26
DDX25	FSHR	IRS4	NKX2-5	PDE3A	RLBP1	SOX2	TPO	UTP14C
DMC1	GALNTL5	LEP	NLRP14	POMC	RNF212	SOX8	TRH	WNT4
DUOX	GCM2	LEPR	NPAS2	PRDM9	SECISBP2	STX2	TRHR	WWOX
DUOX2	GHR	M1AP	NR0B2	PRM1	SLC26A4	TBL1X	TSHB	XRCC2
DUOXA2	GLIS3							

The criteria for inclusion in the presented article were the following ones: 1. The testing method must be next-generation sequencing; 2. The included tests must involve more than two genes; 3. Tests, which involved only karyotyping, searching for Y microdeletions, and array-CGH were excluded.

Information about the number of tested genes, coverage of the panels, turnaround time was collected from the page of GTR, from the home page of the laboratory or by contacting the laboratory. Details about the five most common genes, included in the panels, the coverage of the panels, and information about any additional tests, which were included in the package, for example testing for aneuploidy, Y microdeletions and copy number

variations (CNV) were also gathered. All genes, included in the different panels were tabulated and compared. The company names are not reported to avoid any conflict of interest.

Results

The results from searching the GTR database returned 94 human tests results in total. This included results for laboratories, which provided karyotyping and array-CGH analysis, testing for Y microdeletions, duplication/deletions analysis, etc. All of the results, which did not meet the inclusion criteria, were excluded and in the end there were eleven laboratories, which offered NGS panel testing for male infertility (*Table* 1).

The sensitivity of the panels varied between 95% and 99.8%. Only Laboratory VII did not offer a validated panel with sensitivity and specificity, but suggested a list of genes, associated with male infertility. The shortest turnaround time was 18 days (Laboratory IV), while the longest possible was 63 days (Laboratory VIII) (*Table* 1).

The eleven genetic laboratories tested 230 different genes in total and the genes were available online on the sites of the laboratories. They provided gene testing in their panels or lists for disorders, for which male infertility was a major symptom such as spermatogenic failure, hypogonadism with or without anosmia, cystic fibrosis and congenital absence of vas deferens, endocrine disorders. Also, they had included conditions, for which male infertility was combined with other symptoms and could be part of a syndrome, for example Bardet-Biedl syndrome, Joubert syndrome, nephronophthisis (*Table* 2). However, 65 genes (28.26%) from the different panels were published only in single publications as a reason for male infertility and had an uncertain role for this condition.

The number of genes, included in the different panels, varied significantly from 5 to 136 genes with Laboratory III and Laboratory XI offering the smallest panels, while Laboratory VIII provided the biggest one (*Table* 1). Laboratory VI offered different panels: one for male infertility in general and one for infertility due to the spermatogenic failure. Laboratory V offered one panel, which was considered appropriate for both sexes and included 94 genes. It was called Infertility panel. The Laboratory VII did not offer a default panel, but a list of genes, which could be customized according to the indication of the patient.

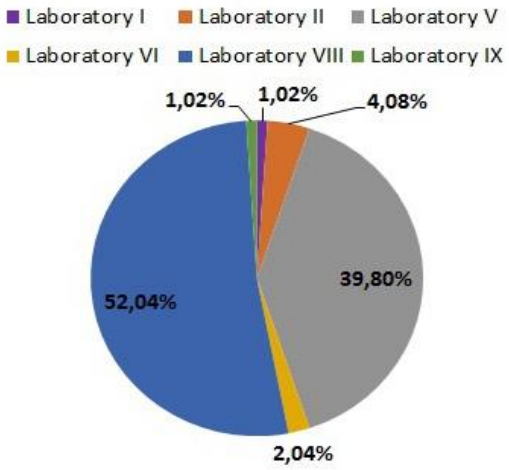

Figure 1 Percentage of genes that is unique to a panel.

We also checked which were the five most common genes, included in the eleven gene sets. *CFTR* was the only gene, suggested by all laboratories. Genetic testing of the Androgen receptor (*AR*) gene was part of testing for male infertility in 10 out of 11 laboratories – it was not on the list only of Laboratory VII. *CATSPER1* is responsible for the production of a protein, needed for the sperm movement [6], and was tested also in 10 of 11 laboratories, the only exception was Laboratory IX. The genes for the receptors for follicle stimulating hormone (*FSHR*) and for the luteinizing hormone/chorionic gonadotropin (*LHCGR*) were supported in 8 of 11 laboratories.

98 genes in total from all 230 genes tested were included only in one panel (*Figure* 1) and we tested only by a specific laboratory. Laboratory VIII had the highest percentage of unique genes tested – 51 (51.52%) genes. Laboratories III, IV, VII and X offered genes, all of which were covered by the other panels. However, of these 98 genes, which were unique, 45.46% had an uncertain role for the male infertility.

Ten of the laboratories (90.91%) offered testing for sex chromosomal aneuploidy, which could be included in the NGS panel as CNV analysis or ordered additionally as cytogenetic analysis. Testing for Y microdeletions was possible in nine (81.82%) of the eleven laboratories, but only in three of them it was included in the panel. In the other six, it was additionally available (*Table* 1).

Discussion

Next-generation sequencing has revolutionized medical genetics with its ability to test for a panel of genes within a single experiment. It is both cost – and time efficient and that is why today it is widely used in the clinics, including in the field of reproductive medicine and male infertility [5, 7]. Besides the great potential of this technology, it has some limitations such as difficulties in covering all regions of the human genome, in detecting mutations in intronic regions or regions, which are rich in guanine-cytosine. Moreover, it is not so successful in detecting large rearrangements such as large deletions or duplications, which could be the reason for male infertility [8, 9]. That is why the results from the NGS still need to be validated by Sanger sequencing and some other conventional methods – array – CGH, Multiplex ligation-dependent probe amplification (MLPA), etc. [7, 10]. NGS also generates a big amount of data, which needs to be further processed. In addition, sometimes the clinicians could be puzzled if they receive a variant of uncertain significance from the NGS and this could represent a great challenge, especially explaining this finding to the patients during the genetic counseling [11].

Despite the certain limitations, NGS and targeted panel testing could be of tremendous help for the clinicians, dealing with male infertility. The panels of the above-described laboratories included genes, such as *AR*, *CATSPER1*, *CFTR*, for which there is certain evidence that they correlate with male infertility [12-14]. *CFTR* gene has been associated lately not only with congenital bilateral absence of vas deferens, but also with non-obstructive azoospermia and severe oligozoospermia [15]. Moreover, the NGS technology has led to discovery of new genes, associated with male infertility. For example, the *DMRT1* gene, which regulates the Sertoli cells, is now considered essential for the normal spermatogenesis, and mutations in this gene could lead to oligospermia [16]. Mutations in another gene, *TEX11*, which is

expressed exclusively in the testicles, could lead to azoospermia [17]. Changes in the genes, responsible for the production of dynein, could lead to asthenozoospermia [18]. Ordering one of the panels for male infertility could provide a rapid diagnosis, improve the patient care and help the couples to conceive. However, due to the lack of guidelines on genetic testing for male infertility it is not clear which of the included genes should be tested first and for which specific subtypes of male infertility are these genes applicable. It is the main responsibility of the clinicians and the genetic counselor to recommend the right NGS panel. The question remains – is it more helpful for the patient to test for more genes, or the bigger number of genes would not lead to higher diagnostic utility.

Testing for chromosomal aberrations, Klinelfeter syndrome and Y microdeletions were not part of most of the above described panels, but were additionally available. However, they are among the most common reasons for male infertility, especially in cases of impaired spermatogenesis [3]. Moreover, karyotyping is recommended before undergoing assisted reproductive treatment. It could be useful not only for detecting the cause for male infertility, but it could also establish a balanced chromosomal rearrangement, thus preventing a potential miscarriage or birth of children with multiple anomalies [19]. Another step before ordering NGS testing for the patient could be analysis for Y microdeletions, since the test is simple and informative, especially in cases of azoospermia or severe oligozoospermia [20]. If no reason for the male infertility is established at this point, the next step could be targeted panel testing. This stepwise approach could be more useful both for the clinicians and for the patients.

Also, due to the specific structure of the Y chromosome and the big percentage of repetitive regions in it, CNV testing could give false-negative results [22]. That is why it is plausible to believe that the NGS panels for male infertility should be at a later stage of the diagnostic process in male patients with infertility.

However, there are no certain guidelines about the target patients and when the clinician should order a testing panel. The genetic abnormalities are very common in cases of azoospermia and panel testing could be the first choice, but in cases of idiopathic infertility, the genetic variant could be a new one, which would require WES or WGS and NGS panel testing would not be the first choice [5]. Moreover, it is not clear which genes, correlating with male infertility, should be included in these panels. For example, the gene for the FSH receptor was included in the panels of 8 laboratories. However, there is controversial data about its role as a factor for male infertility. Three research groups from Italy, Greece and China have published articles denying the role of different polymorphisms in the *FSHR* gene on male infertility and spermatogenic failure [22-24].

Also, there are other genes such as for example *HFE (*included in 3 panels*), IGSF1* (included in one panel), *PRM1* (included in one panel), for which there are publications denying their role in the etiology of male infertility [25-27]. Experimental animals, who lack a normal copy of the *IGSF1* gene demonstrate macroorchidism, but have no fertility issues [26]. Another gene, part of a panel for male infertility – *PROM1*, is usually used as a marker for seminoma, not for infertility [28]. Moreover, it is difficult to establish a new candidate gene for male infertility because even fertile patients, used for healthy controls, might have some problems with the sperm morphology or motility [5]. That is why even though NGS could detect different mutations, it could be difficult to interpret the findings and whether these variants are pathologic or of uncertain significance.

Some of the included genes were responsible for conditions, which typically include other major symptoms and male infertility could be a minor symptom, for example Bardet-Biedl syndrome and nephronophthisis. This raises the question is it necessary to screen infertile males for such conditions, which could be easily recognized and are usually diagnosed at a younger age.

Also, not all of the included genes correlated with certain findings for male infertility. For example, one of the panels included genes, responsible for different types of retinitis pigmentosa. Even though there are publications of mutations in genes, leading to both male infertility and retinitis pigmentosa possibly due to impaired ciliary function, some of these mutations have been reported in single families and are not confirmed by large cohort analysis [29, 30]. Affected individuals with another condition – pantothenate kinase deficiency, which involves retinitis pigmentosa and is included in one of the panels, are usually so severely affected that they die before they can reproduce [31].

Overall, NGS would become an essential part of diagnosing and treating male infertility. The number of the included genes in the panel could vary, but in our opinion *CFTR*, *AR*, *CATSPER1* genes are good candidate genes for every panel, testing for male infertility in general. It is essential for the clinicians to be aware of the limitations of the NGS and of the content of the panel in order to provide the best care for the patient.

Conclusion

Next-generation sequencing could provide a major breakthrough in the process of diagnosing the etiology of male infertility, especially in cases of idiopathic infertility. However, due to the lack of official guidelines about which genes should be included in the panels, the clinician is responsible for choosing the most proper panel for its patients. Moreover, the NGS panel testing does not eliminate the need of karyotyping, and testing for Y microdeletions and CNV. This is why we believe that the clinicians should use a stepwise approach and recommend the NGS panel testing on a second stage of the analytic process. One should also consider that some of the panels include genes, which have a controversial role in the etiology of male infertility. Nevertheless, NGS is cost- and time-efficient and could be extremely helpful when providing the best patient care, especially in cases of idiopathic infertility.

Acknowledgments

Not applicable.

Ethical Issues

For this type of study formal consent is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

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