Personalized Medicine for the Infertile Male

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KEYWORDS

• Male infertility • Personalized medicine • Proteomics • Genomics

KEY POINTS

- Personalized medicine uses a patient's genotype, environment, and lifestyle choices to create a tailored diagnosis and therapy plan, with the goal of minimizing side effects, avoiding lost time with ineffective treatments, and guiding preventative strategies.
- Sir William Osler, one of the founding fathers of Johns Hopkins Hospital, recognized that "variability is the law of life, and as no two faces are the same, no two bodies are alike, and no two individuals react alike, and behave alike under the abnormal conditions we know as disease."[1]
- The study of the -omics: proteomics, genomics, lipomics, and so forth, is yielding an array of new biomarkers to characterize the type of infertility and detect and/or monitor genetic changes, possibly from environmental factors.

INTRODUCTION

Sir William Osler, one of the founding fathers of Johns Hopkins Hospital, recognized that "variability is the law of life, and as no two faces are the same, no two bodies are alike, and no two individuals react alike, and behave alike under the abnormal conditions we know as disease."[1] Centuries later, Sir Osler's hope for personalized medicine is ready to take the center stage in health care. Personalized medicine uses a patient's genotype to tailor further diagnostic testing and therapies to minimize side effects, avoid lost time with ineffective treatments, and guide preventative strategies. The mapping of the human genome in 2003, the discovery of single nucleotide polymorphisms (SNPs) and advances in RNA microarrays have facilitated advancement in the study of the "-omics:"

- Genomics: study of genes and their function
- Proteomics: study of proteins
- Metabolomics: study of molecules involved in cellular metabolism
- Transcriptomics: study of mRNA
- Glycomics: study of cellular carbohydrates
- Lipomics: study of cellular lipids
- Spermatogenesomics: comprehensive study of all factors affecting spermatogenesis

Fig. 1 illustrates the transition from a "one-size-fits-all" approach to that of precision medicine.[2] By considering each patient's unique characteristics, ranging from genetics to lifestyle choices, the goal of personalized medicine is to choose and administer therapy plans in a more rapid and targeted fashion, minimizing the margin for error or failure. Personalized medicine has already made its way from the laboratory bench to the patient's bedside. Four percent to 5% of patients with cystic fibrosis have the G551D mutation in the cystic fibrosis transmembrane conductance regulator gene (CFTR), causing the CFTR-driven ion gate to remain closed, resulting in excess pulmonary mucus production.[3] Ivacaftor is a Food and Drug Administration–approved medication for children as young as 6 months of age with the G551D mutation. In patients with colorectal

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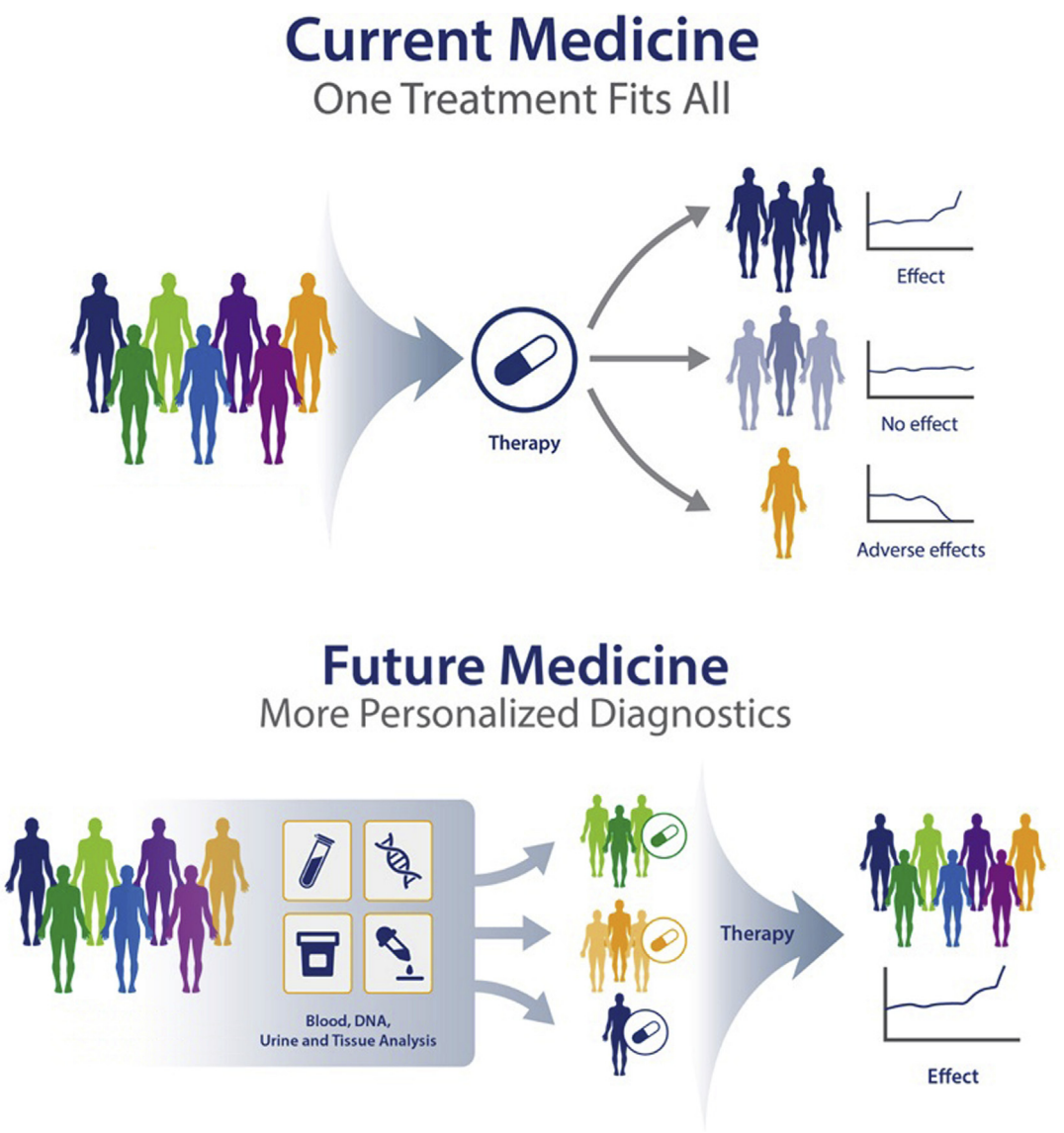

Fig. 1. Comparison of the current model for medicine versus the future personalized model. (*From* Barbeau J. PDX and Personalized Medicine. Crown Bioscience blog. 2018. Available at: https://blog.crownbio.com/pdx-personalized-medicine. Accessed March 13, 2020; with permission.)

cancer, somatic mutations in the PIK3CA gene are associated with improved cancer-specific survival with postoperative aspirin.[2]

Within reproductive endocrinology, preimplantation genetic diagnosis and screening (PGD, PGS, respectively) are widely offered to couples to diagnose or to screen for undesired genetic diseases. The PGD International Society estimates greater than 100,000 cycles have been performed over the last 23 years. Most PGD is for aneuploidy testing, to improve in vitro fertilization (IVF) outcomes in patients with recurrent pregnancy loss, or for those who have failed IVF.[4] In cases of a family history of X-linked diseases, PGD has been used to select for female embryos, to avoid genetic disease in male offspring. In 2004, it was estimated ~1000 children had been born after undergoing PGD/PGS, to at-risk parents, with the same prevalence of congenital malformations as the general population.[5]

Personalized medicine is being promoted and embraced by physicians, politicians, and patients. In 2007, then President Barak Obama created the Genomic and Personalized Medicine Act, providing funding for the advancement of precision medicine. The United States Department of

Health and Human Services also issued a Personalized Health Care directive to increase the usefulness and application of genomic knowledge. With government financial support and new research being published and presented by physicians and scientists every year, patients can expect to benefit from targeted diagnostics, preventative strategies, and more effective therapies.

STANDARD MALE INFERTILITY WORKUP: WHAT IS THE STANDARD MALE INFERTILITY WORKUP

Infertility is defined as an inability to achieve pregnancy after at least 12 months of regular, unprotected intercourse. It occurs in ~15% of couples, with male infertility accounting for the primary or combined cause in 50%.[6] The evaluation of the infertile man includes a thorough history and physical examination to identify abnormalities in the testes, vas deferens, or seminal vesicles, as well as risk factors for infertility, such as a history of cryptorchidism, testicular trauma or infections, or a family history of infertility. Testing begins with 2 semen analyses, performed after 1 to 3 days of abstinence and at least 2 to 3 weeks apart. The World Health Organization[7] has established standard ranges of normal, based on population studies:

- Sperm concentration: ≥15 million sperm per milliliter of semen
- Sperm volume: greater than 39 million
- Semen volume: ≥1.5 mL
- Morphology: greater than 4% normal form
- Motility: ≥40%

Specific abnormalities in the semen analysis, when present in both samples, will prompt varying workups. Moderate oligospermia (concentration ≤10 million/mL) prompts an endocrine evaluation, with a total testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone, whereas severe oligospermia (concentration ≤5 million/mL) requires a karyotype and evaluation for Y-chromosome microdeletion assay. For men without any sperm, or azoospermia, the above tests help to distinguish between obstructive and nonobstructive azoospermia (NOA).

However, the semen analysis is not a perfect test. Couples with normal semen analyses are still unable to spontaneously conceive, and conversely, men with abnormal semen analyses may have normal fertility. Ultimately, the most common diagnosis in male infertility is idiopathic.[8]

When an infertility-related disease is identified, there are few therapies to directly treat the cause of the infertility. Rather, couples rely on assisted reproductive technologies to bypass the cause in favor of the desired result: intrauterine insemination or IVF, with or without intracytoplasmic injection (ICSI). For men without sperm in the ejaculate, sperm is acquired via epididymal or testicular extraction, which may be performed either in the office with local anesthesia or in the operating room under a general anesthetic. However, if sperm cannot be identified, such as in Sertoli cell–only (SCO) syndrome patients, whereby successful sperm extraction is at best 44.5%, as reported by a tertiary-care, high-volume center,[9] couples are left with the options of donor sperm or adoption.

At its core, fertility is the successful combination of the genetic material of a solitary egg and sperm, and then uterine implantation of the embryo. Every step in this process is guided by genetics, and as evidenced by the treatment approach discussed above, male infertility is not well suited for a "one-size-fits-all" approach. Ultimately, this makes the study of male infertility a unique niche for the application of personalized medicine.

SPERMATOGENESIS FAILURE: THE FUTURE FOR SPERMATOGONIA STEM CELL TRANSPLANTATION AND GENOMIC EDITING

Spermatogenesis failure may be endocrine (testosterone production) or exocrine (sperm production) derived. Medical management for endocrine spermatogenesis failure is well established, including supplemental luteinizing hormone, recombinant human FSH, selective estrogen receptor modulators, and aromatase inhibitors.[10] Traditionally, there have been few management options for dysfunctional spermatogonial stem cells (SSC), which are required for self-renewal and differentiation into mature sperm. Examples of this include patients who are status-post chemotherapy and/or radiation, those with maturation arrest, or SCO syndrome, which includes Klinefelter syndrome and AZF microdeletions.[11] Without functional SSCs, patients present with NOA and are dependent on surgical sperm extraction with eventual IVF/ICSI to achieve fatherhood. In addition, patients may require PGD to prevent NOA inheritance in offspring.

Although the above is a solution, it does not correct the root of the problem. Successful spermatogenesis requires spermatogonia proliferation, spermatocyte development, and spermatid differentiation. Failure at any point in this process results in impaired infertility.[12] In a review of testicular biopsy samples in 534 men undergoing infertility evaluation, only 3.2% showed normal histology and spermatogenesis. Maturation arrest

was present in 34% of the patients, hypospermatogenesis in 32%, and SCO syndrome in 16%.[13] Repopulating the testis with functional cells through spermatogonial stem cell transplantation (SSCT), with the added possibility of germline genomic editing to correct for disease-causing mutations, is a burgeoning area of research for spermatogenesis failure.[14]

SSCT has thus far yielded healthy offspring in rodents. Wu and colleagues[15] demonstrated that SSCs, cryopreserved for 12 to 14 years, could be implanted into adult male mice testes, previously treated with alkylating agent busulfan. Two months after transplantation, the number and length of spermatogenesis colonies were equivalent between freshly isolated and cryopreserved cells. Sperm heads were isolated from the recipient testes of cryopreserved SSCs, and ultimately yielded 5 healthy pups, of whom two were produced by natural mating. Furthermore, these pups were fertile and produced fertile generations that appeared normal.

There are several challenges with translating this research to humans. In pediatric oncology patients requiring gonadotoxic chemoradiation therapy, there may be more than 14 years between tissue harvesting and desire for fertility, in which time possible genetic and epigenetic abnormalities may be incurred. Furthermore, there are a limited number of SSCs derived from the small testis biopsies, requiring in vitro expansion before transplantation. Thus far, human SSCs have shown successful propagation in mouse testes, but have yet to be demonstrated in large animal models.[13] SSCT depends on the identification and isolation of normal SSCs, which must survive the freezing and thawing process. Finally, SSCT of flawed SSC may still result in impaired spermatogenesis (ie, maturation arrest), or inheritance of genetic defects that may render future offspring infertile (ie, Y-chromosome microdeletions). However, several advances have been made in the identification of specific genes contributing to male infertility, as well as genomic editing.

Genomic abnormalities affect approximately 15% of infertile patients with azoospermia or severe oligospermia. Currently, these men are recommended a very limited genetic workup, namely a karyotype and testing for Y-chromosome microdeletions, in addition to an endocrine evaluation.[16] This workflow has largely remained the same for more than 15 years, and unfortunately, up to 70% of men will be diagnosed with idiopathic male-factor infertility. Genome-wide association studies have focused on identifying genes that cause or contribute to spermatogenic failure. These candidate genes can then be considered targets for genomic editing. Cannarella[17] created a complete list of 60 genes thought to cause human spermatogenesis failure (**Table 1**). The authors expect the number of target genes to continue to grow. With further multicenter studies, the hope is that these genes will be linked to findings on the semen analysis (ie, asthenospermia, vs globoozospermia, or cryptospermia), which will then narrow the number of genes tested.

If the first step to treating male infertility is to identify the cause, then the next step is to repair the defect. Engineered, site-specific nucleases carry the possibility of genetically modified SSCs, which would overcome fertility obstacles, as well as prevent the passage of inheritable diseases to future generations. Zinc-finger nucleases (ZFNs), transcription-activator-like effector nucleases (TALENs), or the RNA-guided clustered regularly interspaced short palindromic repeat (CRISPR-Cas9 system) generated DNA double-stranded breaks at specific sites. The break may be repaired by error-prone nonhomologous end joining, or by more precise homology-directed repair (HDR), which depends on a repair template of DNA that complements the flanking area of injury, to control what is inserted into the break.[18]

ZFNs were the first platform for genomic editing. Using chimeric proteins and an endonuclease, the zinc finger can bind to ~3 nucleotides, which activates the endonuclease to create a break in the DNA. The break is then repaired as explained above. The ZFN is time-intensive to engineer, and although both halves of the ZFN must recognize the DNA sequence, there is known off-target activity, which has the possibility of disastrous outcomes down the line if used in SSCs.

TALENs use effector proteins from the *Xanthomonas* bacterial species. The DNA binding domain consists of ~34 amino acids, with a key number 12 to 13 amino acid, named the repeat variable dinucleotide (RVD). The RVD is highly variable and changes to it dictate which DNA sequence is bound. In this way, the TALEN is more highly versatile and easily engineered than the ZFN. However, it is still subject to off-target activity and is not as reliable or efficacious as the CRISPR-Cas9 nuclease system.

CRISPR-Cas9 is derived from *Streptococcus pyogenes* and relies on a single-guide RNA (sgRNA) for site-specific DNA recognition and cleavage, and an endonuclease (Cas9). The sequence-specific targeting element (crRNA), which is integrated into the sgRNA, recognizes and pairs with a DNA length of ~20 to 60 nucleotides. The targeting of this area of DNA depends on a protospacer adjacent motif (PAM), which is present in nearly every gene, at multiple sites,

Table 1
Genes involved in human spermatogenic failure

Gene Name	Full Name	MIM Number	Infertility Phenotype	Cytogenetic Location	References
AK7	*Adenylate kinase 7*	615364	Flagella abnormalities	14q32.2	Lores et al, 2018
AURKC	*Aurora kinase C*	603495	Macrozoospermia	19q 13.43	Ben Khelifa et al, 201l
BRDT	*Bromodomain, testis-specific*	602144	Acephalic spermatozoa	1p22.1	Li et al, 2017
CATSPER1	*Cation channel, sperm-associated, 1*	606389	Oligozoospermia	1lql3.1	Avenarius et al, 2009
CCDC39	*Coiled-coil domain-containing protein 39*	613798	Oligoasthenozoospermia. Flagella abnormalities	3q26.33	Ji et al, 2017
CEP135	*Centrosomal protein, 135 kDa*	611423	Flagella abnormalities	4q12	Sha et al, 2017a, b, Tang et al, 2017, Coutton et al, 2018
CFAP43	*Cilia- and flagella-associated protein 43*	617558	Flagella abnormalities	10q25.1	Sha et al, 2017a, b, Tang et al, 2017, Coutton et al, 2018
0CFAP44	*Cilia- and flagella-associated protein 44*	617559	Flagella abnormalities	3ql3.2	Sha et al, 2017a, b
CFAP69	*Cilia- and flagella-associated protein 69*	617949	Flagella abnormalities	7q21.13	Dong et al, 2018
DAZ1	*Deleted in azoospermia 1*	400003	NOA	Yq11.223	Foresta et al, 1999, Mozdarani et al, 2018
DAZ2	*Deleted in azoospermia 2*	400026	NOA	Yq11.223	Foresta et al, 1999, Mozdarani et al, 2018
DAZ3	*Deleted in azoospermia 3*	400027	NOA	Yq11.23	Foresta et al, 1999, Mozdarani et al, 2018
DAZ4	*Deleted in azoospermia 4*		NOA	Yq11.223	Foresta et al, 1999, Mozdarani et al, 2018
DBY (DDX3Y)	*Dead/H Box 3, Y-linked*	400010	NOA (spermatocytes maturation arrest)	Yq11.221	Foresta et al, 2000
DMCI	*Disrupted meiotic Cdna 1, yeast, homolog of*	602721	NOA	22q13.1	He et al, 2018
DMRTI	*Doublesex- andMAB3-related transcription factor 1*	602424	NOA	9p24.3	Lopes et al, 2013, Tewes et al, 2014. Tuttelmann et al, 2018

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Table 1
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Gene Name	Full Name	MIM Number	Infertility Phenotype	Cytogenetic Location	References
DNAAFI	*Dynein, axonemal, assembly factor 1*	613190	Flagella abnormalities	16q24.1	Ji et al, 2017
DNAAF2	*Dynein, axonemal, assembly factor 2*	612517	Asthenozoospermia. Flagella abnormalities	14q21.3	Ji et al, 2017
DNAAF3	*Dynein, axonemal, assembly factor 3*	614566	Flagella abnormalities	19q13.42	Ji et al, 2017
DNAHI	*Dynein, axonemal, heavy chain 1*	603332	Flagella abnormalities	3p21.1	Ben Khelifa et al, 2014, Amiri-Yekta et al, 2016, Wang et al, 2017, Tang et al, 2017
DNAH5	*Dynein, axonemal, heavy chain 5*	603335	Asthenozoospermia, flagella abnormalities	5p15.2	Ji et al, 2017
DNAH6	*Dynein, axonemal, heavy chain 6*	603336	NOA (spermatocytes maturation arrest) Globozoospermia, acephalic spermatozoa	2p11.2	Gershoni et al, 2017, Li et al, 2018a, b
DNAI1	*Dynein, axonemal, intermediate chain 1*	604366	Asthenozoospermia. flagella abnormalities	9p13-p21	Ji et al, 2017
DNAI2	*Dynein, axonemal, intermediate chain 2*	605483	Flagella abnormalities	17q25	Ji et al, 2017
DNAJB13	*DNAJ/HSP40 homolog, subfamily B, member 13*	610263	Flagella abnormalities	11q13.4	El Khouri et al, 2016
DPYI9L2	*DPYI9-like 2*	613893	Globozoospermia	12ql4.2	Koscinski et al, 2011, Harbuz et al, 2011, Ellnati et al, 2012
DYXIC1 (DNAAF4)	*Dynein axonemal assembly factor 4*	608706	Asthenozoospermia, flagella abnormalities	15q21.3	Ji et al, 2017
FANCM	*FANCM gene*	609644	NOA	14q21.2	Kasak et al, 2018, Yin et al, 2018
FS1P2	*Fibrous sheath-interacting protein 2*	615796	Flagella abnormalities	2q32.1	Martinez et al, 2018
HAUS7	*Haus Augmin-like complex, subunit 7*	300540	Oligozoospermia	Xq28	Li et al, 2018a, b

HEATR2 (DNAAF5)	*Heat repeat-containing protein 2*	614864	Flagella abnormalities	7p22.3	Ji et al, 2017
HSF2	*Heat shock-transcription factor 2*	140581	NOA (spermatocytes maturation arrest)	6q22.31	Mou et al, 2013
HYD1N	*Hydrocephalus-inducing, mouse, homolog of*	610812	Asthenozoospermia	16q22.2	Ji et al, 2017
KLHL10	*Kelch-like 10*	608778	Oligozoospermia	17q21.2	Yatsenko et al, 2006
LRRC6	*Leucine-rich repeat-containing protein 6*	614930	Asthenozoospermia, flagella abnormalities	8q24.22	Ji et al, 2017
ME10B	*Meiosis-specific protein with OB domains*	617670	NOA (spermatocytes maturation arrest)	16p13.3	Gershoni et al, 2017
NR5A1	*Nuclear receptor subfamily 5, group A, member 1*	184757	NOA (spermatocytes maturation arrest), oligozoospermia	9q33.3	Bashamboo et al, 2010, Ferlin et al, 2015
P1H1D3	*PIH1 domain-containing protein 3*	300933	Flagella abnormalities	Xq22.3	Paff et al, 2017
PLK-4	*Polo-like kinase 4*	605031	NOA	4q28.l	Miyamoto et al, 2016
RSPH1	*Radial spoke head 1, Chlamydomonas, homolog of*	609314	Flagella abnormalities	21q22.3	Ji et al, 2017
RSPH4A	*Radial spoke head 4A, Chlamydomonas, homolog of*	612647	Flagella abnormalities	6q22.1	Ji et al, 2017
RSPH9	*Radial spoke head 9, Chlamydomonas, homolog of*	612648	Flagella abnormalities	6p21.2	Ji et al, 2017
SEPT 12	*Septin 12*	611562	OAT	I6p13.3	Kuo et al, 2012
SLC26A8	*Solute carrier family 26 (sulfate transporter), member 8*	608480	Asthenozoospermia	6p21.31	Dirami et al, 2013
S0HLH1	*Spermatogenesis- and oogenesis-specific basic helix-loop-helix protein 1*	610224	NOA	9q34.3	Choi et al, 2010, Nakamura et al, 2017
SPATA 16	*Spermatogenesis-associated protein 16*	609856	Globozoospermia	3q26.31	Dam et al, 2007
SPINK2	*Serine protease inhibitor, Kazal-type, 2*	605753	NOA, OAT	4q12	Kherraf et al, 2017
SUNS	*SAD1 and UNC84 domain-containing protein 5*	613942	Acephalic spermatozoa	20q11.21	Zhu et al, 2016

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Table 1
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Gene Name	Full Name	MIM Number	Infertility Phenotype	Cytogenetic Location	References
SYCE1	*Synaptonemal complex central element protein 1*	611486	NOA	10q26.3	Maor-Sagie et al, 2015, Huang et al, 2015
SYCP3	*Synaptonemal complex protein 3*	604754	NOA	12q23.2	Stouffs et al, 2005a, b
TAF4B	*TAF4B RNA polymerase II, TATA Box-binding protein-associated factor*	601689	NOA, oligozoospermia	18q11.2	Ayhan et al, 2014
TDRD6	*Tudor domain-containing protein 6*	611200	OAT	6p12.3	Sha et al, 2018a, b, c
TEX 11	*Testis-expressed gene 11*	300311	NOA (spermatocytes maturation arrest)	Xq13.1	Yatsenko et al, 2015, Sha et al, 2018a, b, c
TEX14	*Testis-expressed gene 14*	605792	NOA	17q22	Gershoni et al, 2017
TEX15	*Testis-expressed gene 15*	605795	NOA	8q12	Okutman et al, 2015, Colombo et al, 2017
TSGA10	*Testis-specific protein 10*	607166	Acephalic spermatozoa	2q11.2	Sha et al, 2018a, b, c
USP26	*Ubiquitin-specific protease 26*	300309	NOA	Xq26.2	Ma et al, 2016
WDR66	*WD repeat-containing protein 66*	618146	Flagella abnormalities	12q24.31	Kherraf et al, 2018
ZMYND10	*Zinc finger mind-containing protein 10*	607070	Flagella abnormalities	3q21.31	Ji et al, 2017
ZMYND15	*Zinc finger mind-containing protein 15*	614312	NOA (spermatocytes maturation arrest)	17p13.2	Ayhan et al, 2014

Abbreviation: OAT, oligoasthenoteratozoospermia.

From Cannarella, R et al. New insights into the genetics of spermatogenic failure: a review of the literature. *Human Genetics* 2019(138):125–140; with permission.

giving the CRISPR-Cas9 nuclease its advantage over TALEN or ZFNs.

Fig. 2 shows the construction of sgRNA and donor DNA, and Fig. 3 illustrates HDR-mediated gene editing.[19] sgRNA are designed to create double-stranded breaks (DSB) within the target DNA (see Fig. 2B), and then single-stranded DNA (ssDNA) oligo insertions are introduced: point (see Fig. 2C), short fusion tag (see Fig. 2D), or a larger fragment of circular DNA (see Fig. 2E). These ssDNA oligos are flanked by 40- to 60-bp sequences on either side, with the required PAM (in this case, 5′-NGG). The 40- to 60-bp sequences are homologous to the sequence surrounding the sgRNA-mediated DSB, thus guiding the ssDNA oligo to the break site for insertion.

In comparison to ZFNs and TALEN, the CRISPR system has been tested on modified SSCs with genome-wide screens, without obvious off-target genetic changes. The system has enabled rapid genomic editing in various species, including correcting the CFTR locus in cultured intestinal stem cells of cystic fibrosis patients.[20] Wu and colleagues[21] demonstrated the ability to apply the CRISPR-Cas9 system to modify the CRYGC gene in SSCs, which were then transplanted into the seminiferous tubules of infertile mice. Subsequent round spermatids were injected into

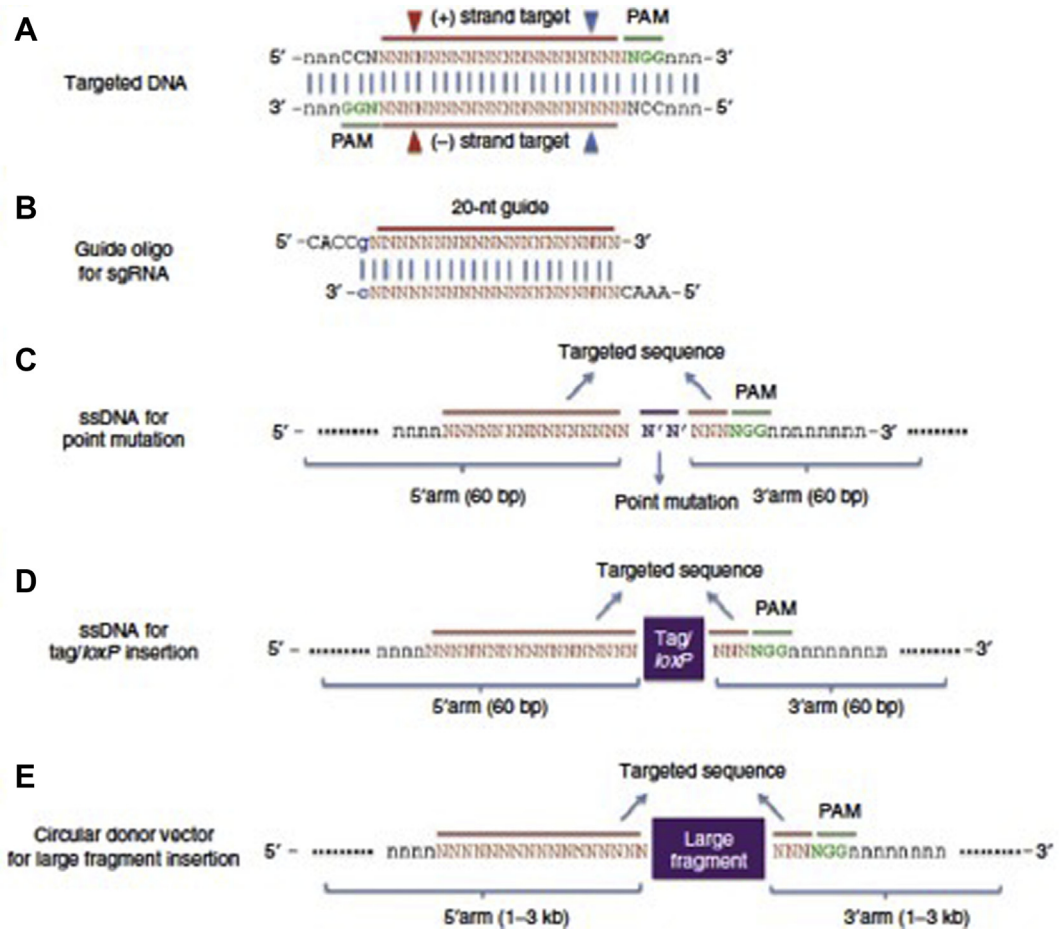

Fig. 2. sgRNA and donor DNA construction. (*A*) Targeted DNA sequence consists of the DNA target (*red bar*) directly upstream of a requisite 5′-NGG adjacent motif (PAM; *green*). Cas9 mediates a DSB ~3 bp upstream of the PAM for the (+) strand (*blue triangle*) or (–) strand (*red triangle*). (*B*) The guide oligos contain overhangs for ligation, a G-C base pair (*blue*) added at the 5′ end of the guide sequence for T7 transcription and the 20-bp sequence preceding the 5′-NGG in genomic DNA. (*C*) ssDNA for point mutation consists of a point-mutation site (*purple*), flanked by 60-bp sequences on each side adjoining the DSBs. (*D*) ssDNA for tag/*loxP* insertion consists of the purple site, flanked by 60-bp sequences on each side adjoining the DSBs. (*E*) A circular donor vector for large fragment insertion consists of a large fragment, flanked by homology arm sequences on each side adjoining the DSBs. (*From* Yang, H et al. Generating genetically modified mice using CRISPR/Cas-mediated genome engineering. *Nature Protocols*. 2014; with permission.)

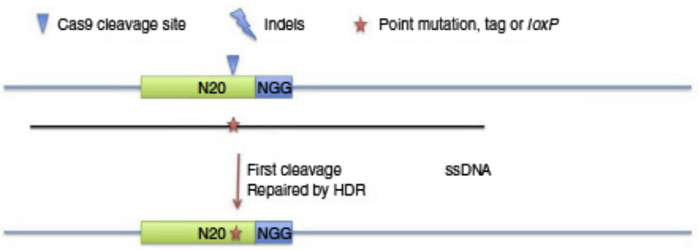

Fig. 3. HDR-mediated gene editing by an ssDNA template at a DSB created by Cas9. (*From* Yang, H et al. Generating genetically modified mice using CRISPR/Cas-mediated genome engineering. *Nature Protocols*. 2014; with permission.)

oocytes, and 100% of the resultant offspring were born with the corrected phenotype. The CRISPR-Cas9 has the advantage of greater efficiency in targeting genomic loci, because several sgRNAs may be used to increase the number of targeted genes.[18]

As referenced earlier in this article, repopulating the testes with a higher density of SSCs is not enough to achieve fertility if the maturation into normal spermatids is not possible. The *c-kit* gene is highly conserved and plays a vital role in male germ cell development. Any mutation in the gene is likely to result in maturation arrest, because of the inability of the SSC to complete meiosis.[22] Yuan and colleagues[22] used TALEN to modify a point mutation in *c-kit*, with resultant normal spermatogenesis from the modified SSCs, which were transplanted back into mouse testes.

Combining the above, men with SSCs, isolated from either prepubertal testis biopsies or during their adult workup for male-factor infertility, have the potential for normal parenthood, through SSC transplantation, with or without correction of genetic mishaps via germline genomic editing. Although the research to support these efforts is still within the laboratory, intensive efforts are underway to progress the science to reality.

SEMINAL PLASMA (PROTEOMICS)

Personalized medicine is not just the search for a therapeutic plan unique to the individual patient. It is also aimed at grouping patients within a spectrum of disease to allow for more targeted diagnostic testing and therapies, with the goal of minimizing the likelihood of ineffective or even harmful treatment. The study of the -omics, or the characterization and quantification of biological molecules, which are central to precision medicine research, is uniquely applied to the field of male infertility, whereby the intricacies of cellular biology, genetics, proteins, and cell signaling are center stage.

Seminal plasma is an easily acquired medium, but one that is rarely studied within male infertility. It is known to contain high levels of lipids, proteins, sugars, and metabolites that intimately interact with the spermatozoa, supporting the acrosome reaction, fertilization, and oocyte interaction.[23] It holds great promise as a possible source of infertility biomarkers, particularly in men with underlying azoospermia. Currently, azoospermia is diagnosed by routine semen analysis, used in the context of a thorough history, physical examination, and endocrine evaluation. To date, however, there are no noninvasive tests to predict the likelihood of zero spermatogenic reserve within the testes.[24] Patients must instead endure a negative testicular exploration. Thus, there has been great interest in identifying a noninvasive biomarker for the diagnosis of NOA secondary to spermatogenic failure.

Seminal plasma contains high concentrations of small extracellular vesicles (sEVs), which contain noncoding RNA, such as microRNA (miRNAs). The miRNAs in particular appear to vary according to the sEVs' cell of origin; therefore, the type of miRNA present within the sEV reflects the pathophysiology of the origin organ.[25] Furthermore, because miRNA are bound to protein complexes and/or contained within the sEV, they cannot cross the blood-testis barrier, making the seminal plasma miRNA a possible biomarker for the pathophysiology of testicular cells.[23] Barceló and colleagues[24] analyzed exosome miRNA levels in seminal plasma from normozoospermic, fertile men, postvasectomy men, men with NOA, and men with severe oligospermia. Of 623 miRNAs that were studied, 1 miRNA (miR-31-5p) was found to have greater than 90% sensitivity and specificity in distinguishing between obstructive azoospermia and NOA, with a greater area under the curve than even plasma FSH (0.957, $P<.0001$ vs 0.85, $P = .004$). With further validation, seminal plasma miRNA is a future tool for urologists to identify men with a real possibility of sperm extraction and recovery for IVF/ICSI.

Protein expression in seminal plasma has also been studied in relation to the level of oxidative stress and reactive oxygen species (ROS). Although elevated ROS has been implicated as a cause of male infertility, data correlating levels of

ROS to pregnancy and live birth outcomes are limited.[15] However, the seminal plasma is rich in molecules that support spermatozoa function; therefore, a greater understanding of the relationship between these proteins and ROS may provide new avenues for male infertility diagnosis and treatment. Using proteomic assays, Sharma and colleagues[26] found proteins unique to men with elevated levels of ROS that were involved in cell morphology, motility, aging, and differentiation, suggesting that these proteins may play a role in apoptosis and necrosis. Wang and colleagues[27] studied the presence of metalloids within the seminal plasma, noting that rising quartiles of seminal arsenic and cadmium were associated with poor sperm motility, whereas a positive correlation was found between seminal zinc and sperm concentration. These studies are examples of novel research using seminal plasma properties, rather than sperm characteristics, to view male infertility, with the ultimate goal being improved diagnostics and more effective therapies.

EPIGENETICS (EPIGENOMICS)

The human genome is made up of DNA, which is a blueprint for proteins to carry out cell functions. The epigenome is a variety of chemical compounds that direct the genome to produce specific proteins. DNA methylation is an epigenetic mechanism, whereby an additional methyl body (CH_3) is added to DNA, ultimately changing that specific gene expression by inhibiting transcription. Expression of noncoding RNAs is another epigenetic mechanism known to contribute to male fertility.[28] Changes to the epigenome are much more dynamic, undergoing changes over years and generations, rather than the human genome, which is relatively stable. Therefore, identification of epigenome-based markers has become an important complement to traditional genetic markers. DNA methylation vastly differs between tissues, but often the disease-specific target tissues are difficult to access or minimally available, such as in the testis. Peripheral blood-based DNA markers have been studied as a close proxy and have already proven useful for cancer diagnosis and prognosis, diabetes, and other diseases.[29]

Two studies have examined peripheral blood-based DNA markers for male infertility. Friemel and colleagues[28] analyzed DNA methylation signatures in 30 infertile and 10 fertile men, using HumanMethylation450 BeadChip. They identified 471 CpG loci that were methylated only in the infertile group, of which 26 did not match to a known SNP. These 26 loci correlated to 15 genes, of which 4 could be linked to male fertility. ENO1 codes for alpha enolase, which has been identified as a marker for sperm fertility in animal studies. MTA2 encodes proteins exclusively expressed by Sertoli cells, and LBX2 and BRSK2 are both expressed in testicular tissue, although their exact role in spermatogenesis remains unclear. The authors chose to focus on 2 other genes, PIWIL1 and PIWIL2, because the piwi-interacting RNA binding proteins are known to have a major role in spermatogenesis. PIWIL 1 controls translation late in spermatogenesis, and PIWIL is required for germline stem cell line renewal. They found the infertile group had substantially higher average DNA methylation rates compared with fertile controls for PIWIL 1 and PIWIL 2: 60% versus 26%, and 80% versus 40%, respectively. With higher rates of methylation, these genes are not expressed; knockout of these genes in mouse models results in infertile mice. Other studies have noted PIWIL2 methylation is associated with lower sperm count[30] and spermatogenic failure.[31]

Sarkar and colleagues[29] supported the above with their own analysis of peripheral blood DNA methylation values in fertile versus infertile men. The investigators identified 170 genes that significantly differed in methylation patterns between the 2 groups, of which 38 played a role in spermatogenesis, including PDHA2, which has a role in sperm energy metabolism, and FHIT, which is involved in testicular germ cell maturation and differentiation. Cross-referencing the Friemel data, there were 52 differentially methylated CpGs (DMCs) in common between the German and Indian study populations. These 52 DMCs represent potential methylation-based markers for male infertility. Ultimately, more studies are needed to validate these findings in other populations.

SUSCEPTIBILITY GENES TO ENVIRONMENTAL FACTORS IN MALE INFERTILITY

There is a burgeoning field of research into the impact of environmental factors on the epigenome. Variations in diet, lifestyle, chemical exposure, and even medications have been linked to major pathologic conditions, such as cancer, obesity, diabetes, and cardiovascular disease. The effect of environmental exposure and lifestyle on reproductive health is still poorly understood but certainly represents an opportunity for personalized medicine in the treatment of male infertility. As previously described, genetic expression is heavily influenced by epigenetic changes, namely histone modifications and methylation patterns. These epigenetic markers are reset at multiple

points from male germ cell development, to the creation of an embryo, and even through puberty.[32] Susceptibility genes are those which are altered by the environment, which may result in disease in the patient or even subsequent offspring.

Men who smoke have been reported to have higher levels of sperm DNA damage,[33] which may result in infertility or recurrent pregnancy loss. Maternal nutrition has been extensively studied and has resulted in creation of prenatal vitamins and other guidelines to minimize risk of infectious disease and neurologic congenital malformations in children. Fewer, if any, guidelines exist for paternal diet. Furthermore, nutrition is known to be tied to epigenetic changes. In studying the agouti viable yellow mouse model, Waterland and Jirtle[34] found that methyl donor supplementation for the gestating mother can directly change the degree of methylation upstream of the agouti gene, thereby changing the pup hair color from salt/pepper to yellow.

Gametic differentially methylated regions are areas within the genome that are not expressed, because of hypermethylation of 1 parental allele. Epigenetic markers, including histone modifications and methylation patterns, are reset at multiple points in the male reproductive cycle, from male germ cell development, to the creation of an embryo, and even through puberty. These changes are susceptible to environmental factors, with higher rates of rare genetic imprinting disease, such as Beckwith-Wiedemann syndrome, or Angelman syndrome, occurring in IVF/ICSI children. Epigenetic changes have been linked to cell culture medium, embryo freezing, timing of embryo transfer, and maternal exposure to high doses of gonadotropins.[35]

In addition to IVF/ICSI, there are many other environmental/lifestyle factors that are thought to impact the reproductive epigenome. Persistent organic pollutants (POPs) were widely used until the 1980s, but are so chemically stable that food supplies continue to be exposed. Many POPs have been found to have a toxic effect on the reproductive and endocrine systems, including sperm quality, spermatic DNA integrity, and hormone levels.[34] Fetal exposures to dibutyl and diethylhexyl phthalates had cumulative and dose-dependent effects of anatomic malformations within the reproductive tract, such as epididymal agenesis and delayed Leydig cell differentiation, cryptorchidism, and hypospadias.

As more of these pathologic gametic differentially methylated regions are identified, the obvious next research area must be treatment focused. Given the vulnerability of male and female reproductive health to environmental factors, nutrition, hormone exposures, and other lifestyle represent opportunities to repair reproductive pathologic condition not only for the patient but also for subsequent generations.

SUMMARY

Both personalized medicine and fertility are anchored in the human genome, and although some parts of precision medicine have much maturing to do within the research laboratory, other aspects have found a place in the infertility clinic, offering options to couples seeking to build their family. The infertility workup has remained largely unchanged and poorly diagnostic. Treatment that gets to the root of the infertility issue is also lacking, with reliance on IVF/ICSI as a workaround to achieving pregnancy. In spermatogenesis failure, SSC therapy may become an opportunity for men with even the smallest amounts of sperm extracted, or with a history of sperm banking, to repopulate the testis, such that their partner may avoid hormonal manipulation in IVF/ICSI. PGD/PGS is well established within infertility clinics, but as CRISPR is further developed, embryos may be pretreated rather than selected, thereby minimizing the risk of IVF/ICSI and embryo harm, and preventing inheritance of the genetic disorder in subsequent generations. Genome-wide association studies are searching for genes contributing to spermatogenic failure. Eventually these genes should be linked to findings on the semen analysis, so the semen analysis can be used as a stepping stone to more directed genetic testing, and eventually therapy, possibly with genomic editing. The study of the -omics: proteomics, genomics, lipomics, and so forth, is yielding an array of new biomarkers to characterize the type of infertility and detect and/or monitor genetic changes, possibly from environmental factors. Seminal plasma and peripheral blood are the new targets of these studies, being the easiest to acquire. Biomarkers within miRNA, DNA methylation levels and locations, and histone changes have been identified, but the data must be validated in different populations. Many research questions remain: will these new biomarker tests be cost-effective? What is the ideal population for these tests? After the tests are validated and widely available, will treatments be available for the diagnoses? What are the ethics involved in genetic testing and editing?

Much like other areas like oncology or immunology, by focusing on the genome, precision medicine is an opportunity to ask more specific questions and tailor therapies in a streamlined

fashion to minimize side effects and ineffective treatments, while producing durable results for patients. With government and industry support, the old protocolized methodology is soon to be replaced.

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