Testing for Genetic Mutations in Severe Testicular Failure Using Commercialized Gene Sequencing Offers Novel Insight for Physicians and Patients

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Introduction

- Non-obstructive azoospermia (NOA) remains a challenging diagnosis for clinicians and patients.
  - Uncertainty in diagnosis, underlying causes.

- Likely genetic mutations predisposing patients to NOA.
  - Challenging to identify due to limited number of patients being tested.

Introduction

- Next generation sequencing has allowed identification of monogenic mutations related to fertility.
  - Over 500 mutations found thus far.
- Commercialization of next generation sequencing now offers a cost-effective approach to perform genetic screens on NOA patients.
- Targeted gene panels offer a focused approach to performing genetic analyses.

Methods

- With IRB approval, deidentified data from infertile men who underwent genetic testing at a single gene sequencing facility were reviewed.

- Two panels offered:
  - Standard panel (>20 publications per gene)
  - Emerging genes panel (<10 publications per gene)

Standard Gene Panel

Standard Genetic Panel	Function
AR	X Chromosome – Encodes the androgen receptor, a key steroid receptor
AURKC	Chromosome 19 – Vital to meiotic cytokinesis, mutations lead to multinucleation
CATSPER1	Chromosome 11 – Sperm-specific calcium ion channel, essential for sperm motility and male fertility
DPY19L2	Chromosome 12 – codes for protein present in human testes vital for spermatogenesis.
FSHB	Chromosome 11 – encodes the beta subunit of follicle-stimulating hormone, key for Sertoli cell proliferation and sperm quality.
FSHR	Chromosome 2 – follicle stimulating hormone receptor, vital for male fertility and sperm production.
LHCGR	Chromosome 2 – Lutenizing hormone and choriogonadotropin receptors, key to propagate LH signal throughout the testicle.
SRY	Y Chromosome – produces sex-determining region Y protein that initiates male sex differentiation.

Emerging Genes Panel

Emerging Genes Panel	Function
CATSPER2	Chromosome 15 – protein key in development of sperm cell flagellum
DAZL	Y Chromosome – homolog in drosophila is essential for spermatogenesis
DDX25	Chromosome 11 – gonadotrophin-regulated testicular RNA helicase important for spermatogenesis
LHB	Chromosome 19 – encodes beta subunit for lutenizing hormone
NR5A1	Chromosome 9 – provides instructions for steroidogenic factor 1, regulating testicular/adrenal development
PRDM9	Chromosome 5 – Involved in transcriptional regulation and essential for meiotic recombination; only expressed in germ cells undergoing meiosis found in the gonads in mouse studies.
PRM1	Chromosome 16 – encodes sperm protamine P1
USP26	X Chromosome – testis specific expression, not present in somatic tissues.

Results

- 37 total patients included.
  - 14 (38%) azoospermic
  - 11 (30%) oligospermic
  - 12 (32%) oligospermic with unspecified sperm density.

- Genetic Mutations Detected
  - 25 patients (68%) tested positive for a mutation in at least one gene.
  - 13 patients (35%) had more than one mutation detected.

- 33 total mutations found
  - 16 (48%) in the standard panel
  - 17 (51%) in the emerging genes panel.

Results – Standard Panel

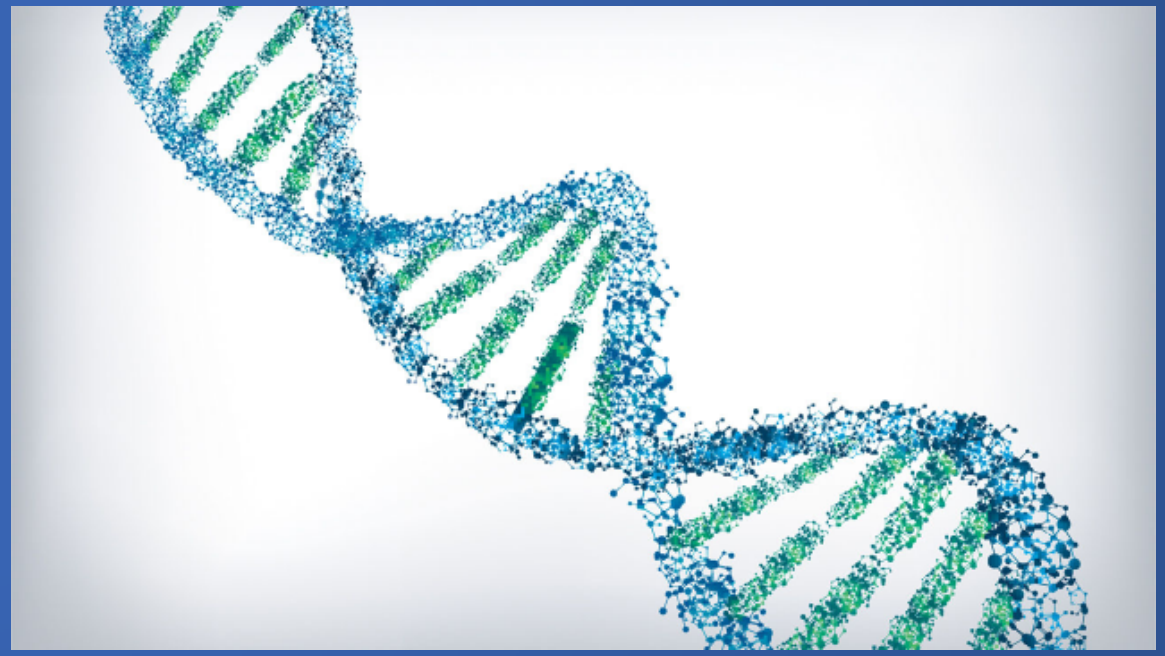
Standard Panel	Azoospermia (n=14)	Oligospermia (n=11)	Unspecified (n=12)
AR	-	-	-
AURKC	-	1	-
CATSPER1	-	-	1
DPY19L2	1	1	1
FSHB	1	-	-
FSHR	1	1	-
LHCGR	1	-	-
SRY	-	-	-
CFTR	5	2	-
TOTAL	**9**	**5**	**2**

Results – Emerging Genes Panel

Emerging Genes Panel	Azoospermia (n=14)	Oligospermia (n=11)	Unspecified (n=12)
CATSPER2	-	2	1
DAZL	1	-	-
DDX25	-	1	2
LHB	-	-	-
NR5A1	-	-	-
PRDM9	4	4	1
PRM1	-	-	-
USP26	-	-	1
TOTAL	**5**	**7**	**5**

Results

- PRDM-9
  - Emerging gene involved in transcription regulation in germ cells
  - 9 total mutations identified
    - 6 indel mutations
    - 3 single-nucleotide polymorphisms



Conclusions

- Significant results are possible using next generation gene sequencing in NOA or severely oligospermic patients.
- The incidence of mutations detected in PRDM9 among the cohort tested was greater than what one would predict for Y chromosome microdeletion testing.
- Impact of these findings must be evaluated:
  - Assisted reproductive technology outcomes
  - Consequences for offspring – genetic counseling?
  - Semen-analysis based testing threshold

Thank you and stay safe!