The genetic causes of male factor infertility: A review

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Introduction

- Infertility : 15% of couples
- genetic abnormalities: 15%–30% of male factor infertility
  - Influencing a variety of physiological processes including *hormonal homeostasis, spermatogenesis*, and *sperm quality*
  - incidence is rising while its etiology remains elusive.
- This paper will discuss the genetic causes of male factor infertility that are considered most relevant today.

Methods

- An exhaustive literature review was performed in PubMed using the keywords "male infertility" and "genetics"
- 646 associated articles → "Y chromosome," "epigenetics," "genomics," "proteomics," and "metabolomics" → 40 articles.
- 1: meta-analysis, 19: original articles, 20: review articles

The Importance of elucidating the genetic basis

- ART (like IVF and ICSI): the frequency of the inheritance of mutations through these procedures and their impact on future generations are not yet fully understood
- ART: majority of children seem normal
- BUT:
  - slight increase in the prevalence of **aneuploidy in the sex chromosomes of ICSI children** (from 0.2% to 0.6%).
  - increased **autosomal chromosome abnormalities** (from 0.07% to 0.4%)
  - difficult to interpret → patients who use ICSI or other ART have a **higher incidence of abnormalities** due to their infertile status

Genetic Causes
(*Chromosomal Abnormalities*)

TABLE 1

Prevalence and phenotypes of common chromosomal abnormalities associated with male infertility.

Genetic abnormality	Phenotype	Prevalence, %
Chromosomal abnormalities	Azoospermia to normozoospermia	5 (total infertile population); 15 (azoospermic)
Klinefelter syndrome	Azoospermia to severe oligozoospermia	5 (severe oligozoospermia); 10 (azoospermic)
Robertsonian translocation	Azoospermia to normozoospermia	0.8 (total infertile population); 1.6 (oligozoospermic); 0.09 (azoospermic)
Y chromosome microdeletions	Azoospermia to oligozoospermia	10–15 (azoospermic); 5–10 (oligozoospermic)
AZFa deletion	Azoospermia, Sertoli cell–only syndrome	0.5–1.0 (2)
AZFb deletion	Azoospermia, spermatogenic arrest	0.5–1.0 (2)
AZFc deletion	Severe oligozoospermia to nonobstructive azoospermia	6–12
Partial AZF-c deletions	From azoospermia to normozoospermia	3–5 (2)

Note: Prevalence listed refers to listed phenotype unless noted otherwise.

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- **Aneuploidy (incorrect chromosome number):**
  - most common error resulting from chromosomal abnormalities in infertile men.
- **Klinefelter syndrome:**
  - most common chromosomal abnormality caused by aneuploidy
  - severe oligozoospermia: 5%; azoospermic: 10%
  - arrest of spermatogenesis at the primary spermatocyte stage
  - nonmosaic, 47,XXY; mosaic, 47, XXY/ 46, XY
  - may try to achieve pregnancy using ICSI
  - **advised :** preimplantation genetic diagnosis (PGD) be performed before ART to ensure that the offspring is not aneuploid

- **Chromosomal translocations:**
  - cause the loss of genetic material at the break points of genes → corrupt the genetic message
  - 4–10 times more likely in infertile males
  - Robertsonian translocations:
    - occur when two acrocentric chromosomes fuse *(**most frequent structural chromosomal abnormalities** in humans)*
    - prevalence: 0.8% = 9 times higher than in the general population
    - variety of sperm production phenotypes from normal spermatogenesis to an inability to produce spermatogonia
  - the risk of passing on the translocation to offspring →FISH, with additional probes added for common translocations

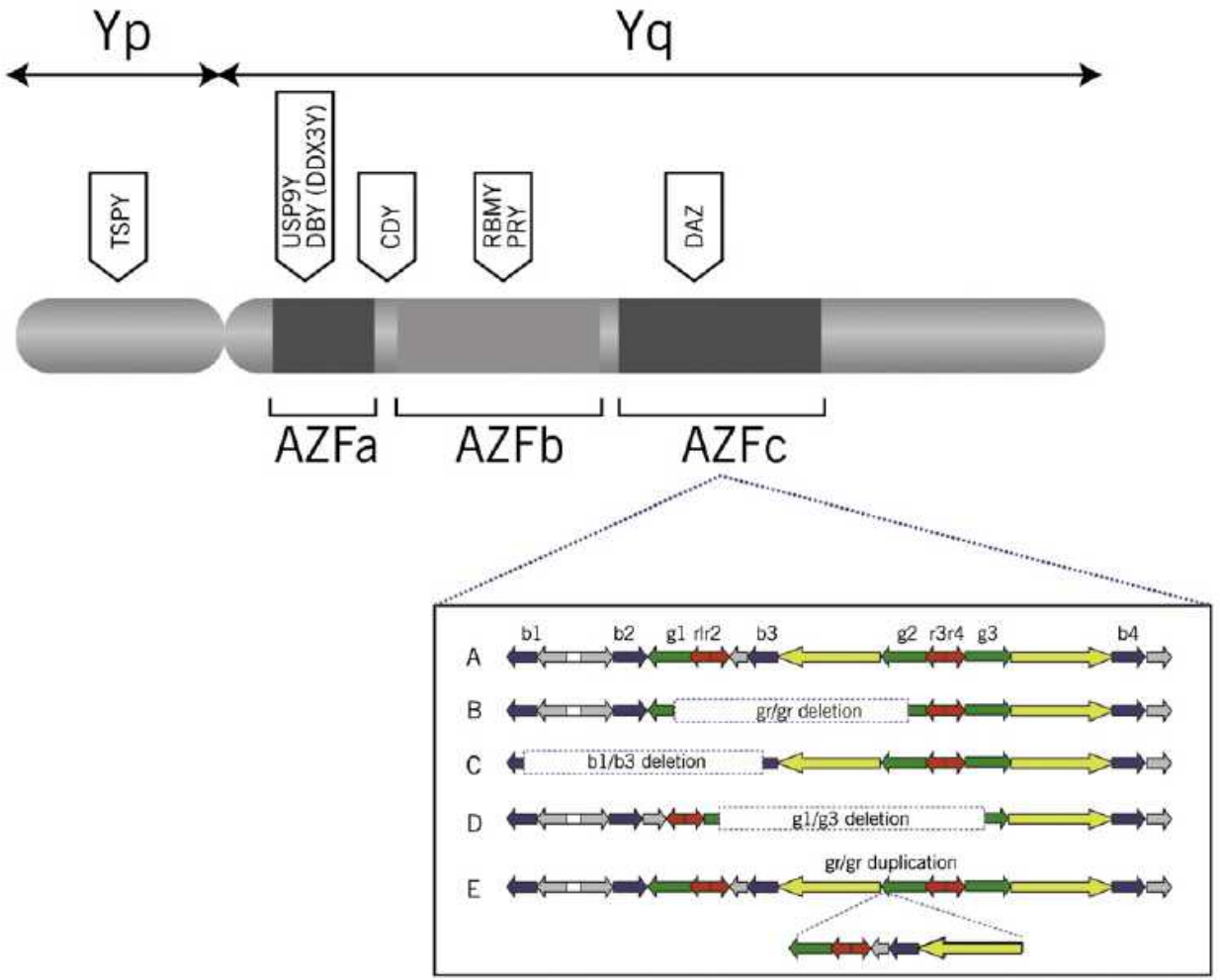
(Y Chromosome)

- Critical for spermatogenesis and the development of male gonads.
- Y chromosome microdeletions: a frequent cause of infertility in males.
- Prevalence: azoospermic (10%–15%), oligozoospermic (5%–10%)
- most frequently occur on the long arm of the Y Chromosome (Yq) → specifically related to failure of spermatogenesis

- A area on Yq is the azoospermia factor region (AZF region): involved in the growth and development of sperm.
- three subregions: AZFa, AZFb, AZFc.
- most common aberrations : multiple gene deletions in the AZFb and AZFc areas → produce a wide range of infertile phenotypes.
- Microdeletions in the AZF region are most often found in azoospermic and oligozoospermic men with normal karyotypes

FIGURE 1

Image of Y chromosome displaying AZF regions and associated genes. Enlarged portion of AZFc region highlights discussed microdeletions. (A) Normal AZFc region; (B) gr/gr deletion; (C) b1/b3 deletion; (D) g1/g3 deletion; (E) gr/gr duplication.



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- **AZFa:** two main genes → USP9Y and DBY (also called DDX3Y)
  - Deletions : remove both of these genes →Sertoli cell–only syndrome (complete Sertoli cells in the testes but a lack of spermatozoa in the ejaculate).
  - DBY: the major gene located in the AZFa region
    - role in infertility : localized in the testis and is involved in the development of premeiotic germ cells
  - USP9Y: also involved in spermatogenesis.
    - Shortening or deletion → azoospermia, oligozoospermia, or oligoasthenozoospermia

- **AZFb:** Deletions cause arrest of spermatogenesis at the primary spermatocyte stage
  - main gene: RBMY
  - A family of PRY genes is also found in the AZFb
    - involved in the regulation of apoptosis (an essential process that removes abnormal sperm from the population of spermatozoa)

- **AZFc:** Deletions produce a wide range of phenotypes
  - reduced spermatogenesis → low sperm concentration
    - nonobstructive azoospermia: 12%
    - severe oligozoospermia : 6%
  - can achieve fertilization with the assistance of ART
  - Studies: only the AZFa and AZFb regions are needed to initiate spermatogenesis → without the AZFc → spermatogenesis will not be completely normal
  - deletion of AZFc → Y chromosome loss →sexual reversal.

TABLE 2

Ethnic variation of gr/gr mutations.

Variation	Effect	Ethnicity	Study
Deletion	Failure of spermatogenesis	Dutch	Repping et al. 2003 (169)
Deletion	Failure of spermatogenesis	Spanish	de Llanos et al. 2005 (170)
Deletion	Failure of spermatogenesis	Italian	Giachini et al. 2005 (171); Ferlin et al. 2005 (172)
Deletion	Failure of spermatogenesis	Australian	Lynch et al. 2005 (173)
Deletion	No correlation	French	Machev et al. 2004 (174)
Deletion	No correlation	German	Hucklenbroich et al. 2005 (175)
Deletion	No correlation	Brazilian	Carvalho et al. 2006 (176)
Deletion	No correlation	Japanese	de Carvalho et al. 2006 (177)
Deletion	No correlation	Chinese	Zhang et al. 2006 (178)
Deletion	No correlation	Sri Lankan	Fernando et al. 2006 (179)
Duplication	Risk for impaired spermatogenesis	Han Chinese	Lin et al. 2007 (52)

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fertility

- The AZFc region also contains genes involved in spermatogenesis.

- DAZ gene :
  - expressed in all stages of germ cell development
  - regulate translation, code for germ cell–specific RNA binding proteins, involved in the control of meiosis and maintenance of the primordial germ cell population
  - Deletions → cause a spectrum of phenotypes ranging from oligozoospermia to azoospermia

- It is critical that azoospermic and severely oligozoospermic men be tested for microdeletions both for accurate diagnosis and genetic counseling before performing ART

- However! the lack of association between testicular phenotype and genotype in affected men forces clinicians to employ inefficient and costly methods, such as polymerase chain reaction (PCR), to determine diagnosis

(Other Genes on the Y Chromosome)

TABLE 3

Genes on Y chromosome with suspected involvement in male factor infertility.

Gene	Location	Reasons for investigation
USP9Y	AZFa	Involved in efficiency of spermatogenesis; deletion or shortening may cause azoospermia, oligozoospermia, or oligoasthenozoospermia
DBY	AZFa	Involved in premeiotic germ cell development
RBMY	AZFb	RNA binding protein/testis-specific splicing factor; reduced expression in azoospermic men
PRY	AZFb	Regulation of apoptosis
DAZ	AZFc	Regulation of translation, meiosis, and germ cell population; codes for RNA binding proteins; reduced expression in azoospermic men; partial deletions related to oligozoospermia
CDY	Yq	Involved in histone replacement
TSPY	Yp	Regulates timing of spermatogenesis; greater copy number in infertile patients

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(Autosomal Gene Mutations and Polymorphisms)

- The CFTR gene (on chromosome 7)
  - mutated in 60%–90% of patients with congenital bilateral absence of the vas deferens (CBAVD) → a form of obstructive azoospermia (disconnection between the epididymis and the ejaculatory duct)
  - ICSI: useful method of FTR mutation (the female does not also carry the CFTR mutation)

- The sex hormone–binding globulin (SHBG) gene, (on chromosome 17)
  - involved in both delivering sex hormones to target tissues and controlling the concentration of androgens in the testis
  - Androgens: important in sexual differentiation and the process of spermatogenesis
  - shorter SHBG:
    - increased levels of spermatogenesis and higher sperm concentration
    - related to elevated levels of circulating SHBG ➔ resulting in higher levels of free androgens to stimulate the spermatogenetic process

- estrogen receptor genes ESR1 and ESR2:
  - association between abnormal spermatogenesis and estrogen insufficiency
  - The promoter region of ESR1 (has a variable number of tandem repeats, (TA)n → related to sperm output:
    - a higher number of repeats on both alleles → with lower levels of spermatogenesis

- The MTHFR (methylenetetrahydrofolate reductase) gene (on the short arm of chromosome 1)
  - codes for an enzyme involved in folate metabolism, a critical factor in DNA methylation and the spermatogenetic process
  - reduced activity → dysregulation of folic acid metabolism → errors in the methylation of genomic DNA and subsequent implications in spermatogenesis
- Mutation of INSL3 gene (insulin-like 3 on chromosome 19) and its receptor LGR8 → related with cryptorchidism

(X-Linked Genes)

- Many expressed in the testis → involved in gametogenesis
- The androgen receptor (AR) gene (long arm of the X chromosome) → plays a role in meiosis and the conversion of spermatocytes to round spermatids during spermatogenesis
- Mutations → androgen insensitivity syndrome (impede the ability of androgens to bind receptor → decreased transactivation potential)
- Kennedy syndrome: possible result of mutations in the AR gene → neurodegenerative disorder

- The AR gene has two polymorphisms: CAG and GGC polymorphisms
- The CAG polymorphism has been studied more intensively than the GGC polymorphism.
- Longer lengths of the CAG polymorphism →decreased transcriptional activity of the AR gene in infertile men (longer polyglutamine tracts are related to male factor infertility)
- Some researchers: shorter CAG polymorphisms were related to higher quality sperm and increased levels of spermatogenesis

- The USP26 gene:
  - the long arm of the X chromosome
  - expressed throughout the testes in the preliminary stages of spermatogenesis
  - involved in histone removal during spermatogenesis and the breakdown and reformation of proteins
- The TAF7L gene
  - expressed in the testis
  - related to the autosomal TAF7 gene, which is a transcription factor → play integral roles in spermatogenesis

- Kallmann syndrome (KS) : cause infertility in males and has both X-linked and autosomal genetic components.
  - defined as idiopathic hypogonadotropic hypogonadism (IHH) combined with anosmia or hyposmia
  - The absence or low levels of sex steroids inhibit or stunt sexual development and spermatogenesis in males.
  - cognitive impairments of sexual development, ocular abnormalities, midfacial clefting, and renal agenesis

EPIGENETIC ERRORS

- Spermatogenesis is a complex series of events vulnerable to the accumulation of errors that can severely affect the spermatogenic process
- Epigenetics refers to alterations of the genetic code that do not affect the basic DNA sequence, such as the addition of different molecules to the DNA, which changes the regulation of transcription and, consequently, gene expression

- the sperm makes to the embryo is a functional centrosome → involved in the process of fertilization, the separation of chromosomes, and cell division
- Rawe et al. : abnormal centrosome morphology and sperm aster formation had difficulty fertilizing oocytes → if a pregnancy was achieved, the fetus was aborted
- Sperm harvested from the testicles before maturation may not have a fully functional centrosome → problems with the segregation of chromosomes and result in a mosaic or aneuploid embryo

- Histones : important contributor to the transmission of epigenetic information.
- Histone markers signify DNA imprinting control regions during the formation of spermatozoa
- The transcriptional control of gene expression is regulated by the addition of acetyl, methyl, ubiquitin, and phosphate groups to histones
- Abnormally modified histones are probable candidates for impeding normal embryogenesis, and their role in the fertility is currently under investigation

- Imprinting, the methylation of DNA, determines which genes from the parental and maternal genomes are expressed in the embryo and is critical for normal development.
- The control of methylation may also be a point at which dysregulation could occur.
- Studies in knockout mice for DNA methyltransferases produced males that were oligozoospermic; however, this has not been replicated in humans

- The correlation between the incidence of imprinting disorders and ART in men with abnormal sperm is a controversial topic.
- Two independent groups reported:
  - increased incidence of *Angelman syndrome* *(rare neurological disorder characterized by cognitive defects, seizures, uncontrolled limb and body movements, spontaneous laughter, and difficulties with speech development)* in offspring from ICSI procedures
  - the incidence of *Beckwith-Wiedemann syndrome* *(large fetal and organ size, hypoglycemia, midline abdominal defects, facial moles, and enlarged tongues)* was almost 5% in children conceived by ART in comparison with an incidence of less than 1% in the general population

- Telomeres : as potential candidates for the production of infertile phenotypes.
- Telomeres protect the genetic information encoded on the chromosome, localize the chromosomes in the nucleus, and play a role in DNA replication
- Abnormal shortening → male factor infertility
- Hemann et al. : telomere length in knockout mice for telomerase (maintains the length of telomeres) → a mechanism that degrades spermatocytes with reduced telomere length to prevent their maturation

- Mitochondrial DNA (mtDNA) inheritance may also impact male factor infertility.
- Abnormal mitochondria cause problems in sperm motility because of aberrations in the mitochondrial sheath
- Passing mtDNA abnormalities to offspring using ART such as ICSI because the entire sperm is injected into the oocyte and the mtDNA of the sperm is conserved.
- oligozoospermic males have been found to have higher rates of mtDNA mutations

NOVEL TECHNOLOGIES

- Adopting a global approach to the examination of novel genes may allow for a more complete understanding of the interaction between genetics and fertility and could uncover genes with unknown roles in infertility
- Incorporating techniques such as genomics, proteomics, and metabolomics into infertility research could assist in creating a complete portrait of the genes involved in infertility and would allow for improvements of ART for the development of more targeted solutions.

- Microarrays are valuable tools for the identification of gene expression profiles of infertile phenotypes
- Microarray technology is also useful in the examination of spermatogenesis.
- Genomic analysis can also be used to determine differentially transcribed genes
- Additionally, microarrays can be used to study the effect of hormones or growth factors on gene expression profiles

- Advantages of using microarrays are that it is a noninvasive test and it is very effective in studying germ cells
- Disadvantages of genomics are that gene expression can vary between two different samples and infertile patients might have pockets of gene expression that are difficult to detect using microarrays

- Proteomics allows for the determination of protein expression profiles of fertile and infertile men.
- Proteins are identified with two-dimensional electrophoresis and mass spectrometry techniques, and the results are used to create maps of the proteome

- The identification of protein biomarkers for male factor infertility will allow for unbiased comparison between fertile and infertile males and will clarify the pathophysiology of the disease
- An advantageous characteristic of genomic and proteomic technology is that the results can be confirmed through replication using other techniques such as Western blots, flow cytometry, and PCR.

- Metabolomics is another emerging area of research in the evaluation of the role of genetic factors in male factor infertility.
- Mass spectroscopy, nuclear magnetic resonance spectroscopy, and other chromatography methods can be used to create profiles of metabolites
- metabolomics has been used to identify biomarkers for oxidative stress (OS), which signal semen quality
- These novel technologies hold promise for advances in the ways in which information about genetic profiles can aid infertility patients.

CONCLUSION

- Although much work still must be completed to fully determine the involvement of genes in the production of infertile phenotypes, current research findings suggest that accurate transmission of genetic and epigenetic information is essential for fertility

Thanks for your attention!