Review Article

# Genetics of Male Infertility – Present and Future: A Narrative Review

*Digumarthi V.S. Sudhakar[1], Rupin Shah[2], Rahul K. Gajbhiye[3]*

[1]Department of Gamete Immunobiology, ICMR-National Institute for Research in Reproductive Health, [2]Lilavati Hospital and Research Centre, [3]Clinical Research Lab and Andrology Clinic, ICMR-National Institute for Research in Reproductive Health, Mumbai, Maharashtra, India

**Abstract**

Infertility affects 8%–12% of couples worldwide with a male factor contributing to nearly 50% of couples either as a primary or contributing cause. Several genetic factors that include single-gene and multiple-gene defects associated with male infertility were reported in the past two decades. However, the etiology remains ambiguous in a majority of infertile men (~40%). The objective of this narrative review is to provide an update on the genetic factors associated with idiopathic male infertility and male reproductive system abnormalities identified in the last two decades. We performed a thorough literature search in online databases from January 2000 to July 2021. We observed a total of 13 genes associated with nonobstructive azoospermia due to maturation/meiotic arrest. Several studies that reported novel genes associated with multiple morphological abnormalities of the sperm flagella are also discussed in this review. *ADGRG2*, *PANK2*, *SCNN1B*, and *CA12* genes are observed in non-*CFTR*-related vas aplasia. The genomic analysis should be quickly implemented in clinical practice as the detection of gene abnormalities in different male infertility phenotypes will facilitate genetic counseling.

**Keywords:** *Azoospermia, genes, male infertility, multiple morphological abnormalities of the sperm flagella, mutations, nonobstructive azoospermia, obstructive azoospermia*

## Introduction

Infertility affects 8%–12% of couples worldwide with a male factor contributing to nearly 50% of the couples either as a primary or contributing cause.[1] East Asia and West Africa harbor the highest numbers of infertility cases.[2] Male infertility is primarily diagnosed based on the evaluation of semen parameters. Nonobstructive azoospermia (NOA) is the most severe form of male infertility that is characterized by a complete absence of spermatozoa in the ejaculate.[3] Other phenotypes of male infertility include asthenozoospermia (reduced sperm motility), oligozoospermia (reduced sperm count), and teratozoospermia (reduced percentage of spermatozoa with normal morphology). Often, infertile men present themselves with abnormalities in multiple semen parameters such as asthenoteratozoospermia that results in reduced or no sperms with normal motility and morphology.

Spermatogenesis is a highly complex process that occurs through successive mitotic, meiotic, and postmeiotic phases involving several molecular pathways. Human spermatogenesis requires an orchestrated expression of a multitude of genes and involves dynamic transcription of over 4000 genes in various germ cell subtypes.[3] Owing to the complexity of human spermatogenesis, male infertility is highly complex with extremely heterogeneous phenotype presentations among infertile men. It is currently estimated that known genetic factors such as chromosomal abnormalities, aneuploidies, Y chromosome microdeletions, and single-gene defects are responsible for at least 15%–30% of male infertility.[3,4]

**Received:** 09-08-2021 **Revised:** 25-08-2021
**Accepted:** 02-09-2021 **Published:** 28-09-2021

Access this article online
Quick Response Code:
Website: www.jhrsonline.org
DOI: 10.4103/jhrs.jhrs_115_21

***Address for correspondence:*** Dr. Rahul K. Gajbhiye, Scientist D & DBT Wellcome India Alliance Clinical & Public Health Intermediate Fellow, Clinical Research Lab and Andrology Clinic, ICMR-National Institute for Research in Reproductive Health, Mumbai, Maharashtra, India.
E-mail: gajbhiyer@nirrh.res.in

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow_reprints@wolterskluwer.com

**How to cite this article:** Sudhakar DV, Shah R, Gajbhiye RK. Genetics of male infertility – Present and future: A narrative review. J Hum Reprod Sci 2021;14:217-27.

Several studies have identified additional genetic factors that include single-gene and multiple-gene defects that are associated with male infertility in the past two decades. However, the etiology remains obscure in a majority of infertile men (~40%), and identification of novel genetic factors linked with idiopathic male infertility is a major research concern.[4] This review, therefore, provides an update on the genetic factors associated with idiopathic male infertility that were identified in the last two decades.

## Objective

To provide a comprehensive update of the genetic factors associated with idiopathic male infertility that were identified in the last two decades. We mainly focused on monogenic causes of isolated or idiopathic male infertility and reproductive system abnormalities in males.

## Search Methods

We performed a thorough literature search using online databases such as PubMed, Embase, Web of Science, Scopus, Google Scholar literature database, and Science Direct [Figure 1]. Articles were searched using search terms related to "male infertility" in combination with the other words that include "genetics", "Y chromosome microdeletions", "exome", "genomics", "genetics", "sequencing", "whole-exome sequencing", "whole-genome sequencing", "next-generation sequencing (NGS)", "azoospermia" "spermatogenesis", "monogenic causes", "genetics of vas aplasia", etc. Further, the quality and the extent of all the evidence supporting selected genes were carefully evaluated manually. We also assessed the experimental quality, patient phenotype assessment, and functional evidence to establish genotype–phenotype correlation using *in vitro* human cell lines and *in vivo* animal models. Candidate genes/genetic factors with significant impact on male fertility and validated by multiple studies were mainly selected for discussion. Articles published between January 2000 and July 2021 were reviewed.

## Male Infertility and Genetic Association

Male infertility is subclassified into four major etiological categories: (a) spermatogenic quantitative defects; (b) ductal obstruction or dysfunction; (c) hypothalamic–pituitary axis disturbances; and (4) spermatogenic qualitative defects.[5] The genetic factors are known to be responsible for approximately 15% of male infertility.

Idiopathic oligoasthenoteratozoospermia (OAT) is the most common form of male infertility followed by azoospermia mainly due to primary testicular failure that manifests as quantitative defects of spermatogenesis. The other common etiologies of male infertility include obstruction or morphological abnormalities of ducts. The other two phenotype categories include

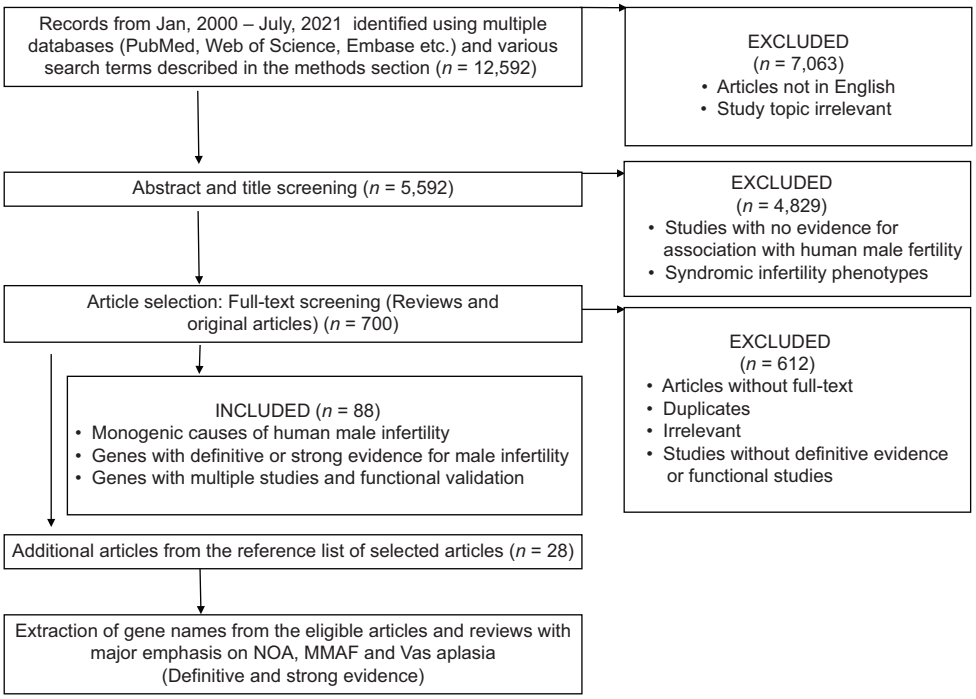


**Figure 1:** Flowchart of review methodology. PubMed and other databases were searched for all articles containing genetic studies on human male infertility as described in the methods limiting to studies published after January 2000. NOA- Non obstructive azoospermia, MMAF -multiple morphological abnormalities of the sperm flagella

perturbed hypothalamic–pituitary axis that results in secondary testicular failure and qualitative defects in spermatogenesis.[5,6] In a routine diagnostic workup, genetic diagnosis is only possible in about 20% of infertile men.

## Chromosome Number and Structural Anomalies

It is a routine practice in infertility clinics to observe for any cytogenetic abnormalities as the first step of choice, in idiopathic infertile individuals. Chromosomal abnormalities range from 5% to 15% in infertile men with the highest prevalence being in men with complete absence of spermatogenesis.[7] Aneuploidy is one of the most common chromosomal abnormalities in infertile men and is generally associated with NOA.[8] Klinefelter syndrome with 47,XXY karyotype or its variants is the most common aneuploidy and is seen in about 14% of azoospermic men.[9] Klinefelter syndrome has an estimated frequency of 1 in 7 among NOA men.[10] Affected individuals typically have small testis and consequent spermatogenic failure. Another numerical chromosomal aneuploidy is 47,XYY, which is rare and men with this karyotype may have normal fertility, but with an increased likelihood of infertility compared to normal 46,XY males.

Apart from chromosomal numerical aberrations, structural aberrations such as deletions, duplications, inversions, and translocations are commonly seen chromosomal abnormalities in about 5% of infertile men.[11] Robertsonian translocations, where long arms of two acrocentric chromosomes fuse to form a long chromosome with a single centromere, are seen in 0.8% of infertile men. The incidence is predominantly seen in oligozoospermic men compared to azoospermic men.[12] The most commonly observed Robertsonian translocations are der (13;14) and der (14;21), of which der (13;14) is predominant.[13] Couples seeking *in vitro* fertilization must be counseled for preimplantation genetic screening because any chromosomal structural abnormalities in the sperm may increase the risk of aneuploidies, unbalanced chromosomal translocations, and imprinting disorders (Robertsonian translocations) in the fetus.[4] Although considered to be a variant of normal karyotype, Chromosome 9 pericentric inversion; inv (9) (p11q12) is another frequent and interesting chromosome rearrangement. Mozdarani *et al*. reported Chromosome 9; inv (9) (p11q12) in 4.69% of infertile men and noticed that the incidence of inversion 9 in infertile men is significantly higher than that of the normal population.[14] Another recent study has reported a novel pericentric inversion inv (9) (p23q22.3) in infertile individuals from Southeast Europe.[15] Despite being benign, pericentric inversions may affect the reproductive outcomes of carrier males by increasing the risk for chromosome abnormalities in live-born offspring, miscarriages, and fertility issues.[15]

## The Human Y Chromosome

The Y chromosome is the smallest human chromosome, which contains 60 million nucleotides and is 57 Mb in size.[16] The distal ends of both *P* and the q arms comprise pseudoautosomal regions (PARs) which recombine with the X chromosome during meiosis. The chromosomal region outside the PARs is not involved in recombination, hence termed as a nonrecombining region of Y (NRY), which comprises 95% of the Y chromosome.[17] However, the NRY flanked on both sides by recombining PARs is better termed as a male-specific region of the Y chromosome (MSY), with 156 known transcription units, 78 protein-coding genes, massive palindrome sequences, and testis-specific genes.[18,19] MSY region has twelve gene families with different copy numbers ranging from two (*VCY, XKRY, HSFY,* and *PRY*) to three (*BPY2*) to four (*CDY, DAZ*) to six (*RBYM*) to approximately 35 (*TSPY*).[20] This copy number may vary among different human populations. The distal part of the long arm (Yq) harbors heterochromatin, whereas euchromatin (~23MB) that contains genes and repetitive sequences spans Yp and the proximal part of Yq [Figure 2].

## Y Chromosome Microdeletions and Male Fertility

Y chromosome microdeletions are small chromosomal deletions that are usually submicroscopic (<5 Mb) and escape detection by normal karyotyping. Tiepolo and Zuffardi (1976) identified *de novo* deletions on the Y chromosome long arm (Yq) in azoospermic men that prompted them to predict the existence of indispensable genes on Yq that are essential for normal spermatogenesis.[21] Later, the entire gene cluster in the distal region of the Y-chromosome long arm was named as azoospermia factor (*AZF*) region. Vogt *et al*. and Skaletsky *et al*. defined the *AZF* region and identified genes essential for male fertility in this region.[19,22] The *AZF* region in the long arm of Y chromosome is further classified into *AZF*a (792 kb), *AZF*b (6.2 Mb), and *AZF*c (4.5 Mb) regions.[23,24] *AZF*a, *AZF*b, and *AZF*c regions harbor several genes which are either exclusively expressed or enriched in the testis and thus likely to have a potential role in spermatogenesis.[25] The presence of repeated homologous sequences in the boundaries of *AZF* regions predisposes these regions to duplication (s) or deletion (s) through nonallelic homologous recombination (NAHR).

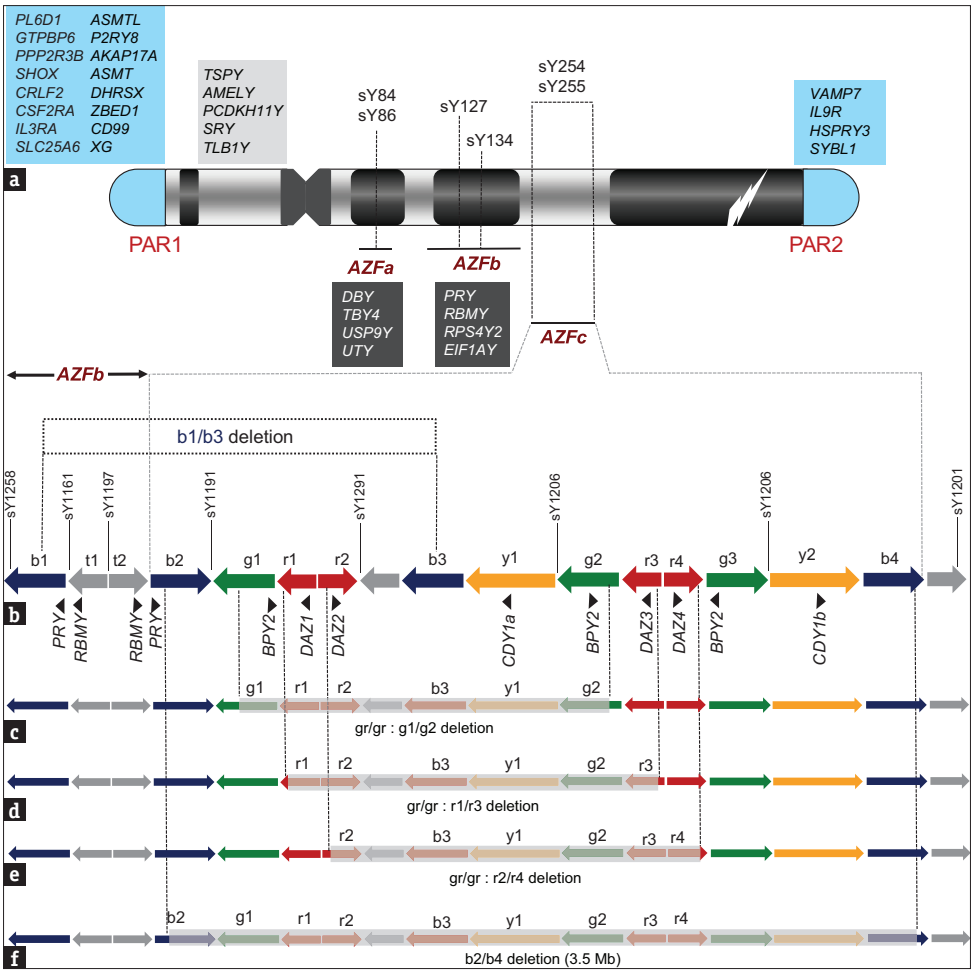


**Figure 2:** Schematic representation of Y chromosome depicting azoospermia factor regions and types of microdeletions. (a) Schematic diagram showing different regions of Y chromosome and genes located in nonazoospermia factor regions; (b) schematic diagram showing the location of inverted repeats, STS markers and b1/b3 deletion in the AZFc region; (c) schematic diagram showing g1/g2 (gr/gr) deletion region; (d) schematic diagram showing r1/r3 deletion (gr/gr) region; (e) schematic diagram showing r2/r4 deletion (gr/gr) region; (f) schematic diagram showing 3.5 Mb complete AZFc deletion (b2/b4 deletion)

Approximately 7% of infertile men show Y chromosome microdeletions worldwide. Among the infertile men, 55% of men with maturation arrest and Sertoli cell-only syndrome have Y chromosome microdeletions.[26] Several studies have shown that microdeletions in *AZF*c region are most common (maximum of 80%), followed by *AZF*b (maximum to 5%) and *AZF*a (maximum to 4%).[27] Although microdeletions are usually common in azoospermic men (16.90%), they are also seen in oligozoospermic men.[28] The prevalence of microdeletions in the *AZF* region may vary among infertile men with different ethnic backgrounds. Approximately 8.5% of Indian azoospermic men show *AZF* deletions, of which *AZF*a deletions alone are 17.2%, *AZF*c deletions alone are 24.1%, and the remaining are combinations of *AZF*a/*AZF*b or *AZF*b/*AZF*c or *AZF*c partial deletions.[29] A more recent study has shown a high frequency of NAHR-mediated deletions in about 25.8% of Indian infertile men (13.1% partial and 6.9% complete *AZF*c deletions, 3.5% *AZF*b deletions, and 2.3% *AZF*bc deletions).[30] Long-term geographical isolation and strict endogamy practice among Indian populations might likely have contributed to the high frequency of deletions among Indian infertile men.[30]

During routine diagnosis for Y chromosome microdeletions using polymerase chain reaction, it is difficult to screen the entire *AZF* region. Hence, several unique sequence tagged site (STS) markers from each region of *AZF* (*AZF*a, *AZF*b, and *AZF*c) are routinely examined.[31] STS markers such as sY84, sY86, DFFRY, sY83, sY740, sY746, sY741, sY742, and sY615 are located in the *AZF*a region; sY98, sY110, sY100, sY80, sY1211, sY143, sY127, and sY134 are located in *AZF*b region. Markers sY152, sY148, sY156, sY581, sY247, sY254, and sY255 are located in *AZF*c region. The markers routinely used for molecular diagnosis may vary based on the ethnic background of the infertile men. The European Andrology Association (EAA) recommends a set of STS markers

for routine Yq deletion analysis which includes sY84 and sY86 (*AZF*a); sY127 and sY134 (*AZF*b); and sY254 and sY255 (*AZF*c).[32] However, an additional set of STS markers have been recommended for Indian infertile men as the EAA-recommended markers did not show deletions in *AZF*a and *AZF*b regions. Thus, non-EAA markers sY746 and sY82 in *AZF*a; sY121, sY128, and sY130 in *AZF*b; and sY145 and sY160 in *AZF*c should be included in regular Yq microdeletion analysis in Indian infertile men to increase the chance of identifying *AZF* microdeletions.[29]

## Partial *AZFc* Deletions

*AZF*c is the best-studied region of the Y chromosome, and deletions in this region are the most common known cause of spermatogenic failure. *AZF*c is located at the distal end of deletion interval 6 (subintervals 6C-6E) on the Y chromosome [Figure 2]. *AZF*c, which spans 4.5 Mb, is well known for its structural complexity and has the largest known palindrome in any genome sequenced to date. Around 95% of the *AZF*c is composed of amplicons (identical sequences).[33,34] The presence of multiple repeated palindromes makes the *AZF*c region susceptible to deletions and thus the *AZF*c region is the most vulnerable to deletions compared to *AZF*a and *AZF*b regions.[34] In addition to extensive array of pseudogenes, *AZFc* region also has protein coding multiple gene families such as *BPY2, CDY1, TTY3, TTY4,* and *DAZ* etc.

Some infertile men show a 3.5 Mb deletion of the entire *AZF*c region (b2/b4 deletion) that harbors 12 multiple copy number genes and transcriptional units. In the routine molecular diagnosis of male infertility, complete *AZF* deletions have a demonstrated prognostic value. However, partial deletions in *AZF*c region are also commonly encountered during routine screening of Y chromosome microdeletions, but the clinical relevance remains speculative.[26,30] The two major *AZF*c partial deletions include gr/gr (1.6 Mb, identified by the deletion of STS marker sY1291) and b1/b3 (1.6 Mb, identified by the deletion of STS markers sY1291, sY1191, sY1197, sY1161, and sY1291) [Figure 2].[35] The other two rare partial *AZF*c deletions which result from gr/gr deletion followed by inversion or vice versa are b3/b4 (deletion of 1.6 Mb and b3/b4 inversion) and b2/b3 (deletion of 1.8 Mb and b2/b3 inversion). All the partial deletions (except b1/b3) only alter gene copy numbers without eliminating entire gene (s) within the *AZF*c region.

### gr/gr Deletions

The most common *AZF*c partial deletion observed is gr/gr deletion that is further subdivided into g1/g2, r2/r4, and r1/r3 based on the recombination pattern between g1-r1-r2 and g2-r3-r4 [Figure 2]. Studies have shown that ethnic variation exists in gr/gr deletions due to specific haplogroup backgrounds and heterogeneity in the gene copy deletions.[35,36] A large study by Repping *et al*. had shown that men with spermatogenic failure had significantly higher gr/gr deletion frequency (3.8%) compared to fertile men (2.2%).[37] Furthermore, recent meta-analyses and a population-based survey of 20,000 Y chromosomes showed that gr/gr deletion is associated with male infertility risk, and infertile men with gr/gr deletions had lower sperm counts compared to fertile men with gr/gr deletions.[27,38,39] On the contrary, some other groups have reported that gr/gr deletion is not useful in predicting impaired spermatogenesis.[40-42] A recent study on the Indian population had shown that the gr/gr deletions are more frequent among oligozoospermic (11.4%), followed by azoospermic (4.6%) and oligoteratozoospermic (2.1%) men compared to the control group (1.53%). Therefore, gr/gr partial deletion is a significant risk factor for low sperm counts in Indian idiopathic infertile men.[30] The study had also shown that the haplogroup is not useful in risk assessment among Indian infertile men. Another study on the Indian population reported an association of gr/gr deletions with low sperm count and gr/gr deletions showed the highest frequency (5.84%) compared to other *AZF*c partial deletions among infertile men.[27] Thus, there is a significant association of gr/gr deletions with impaired spermatogenesis in the Indian population. However, gr/gr deletion frequencies may vary among different ethnic groups among Indian infertile men.

## X-linked Genes and Male Infertility

Men inherit a single X chromosome and are hemizygous for X-linked genes. Earlier, genomic studies had shown that X chromosome is enriched with spermatogenesis genes.[43] Various X chromosomal genes that are linked with fertility in males have been identified in recent times. Wang *et al*. had reported that X chromosome has several genes that play a predominant role in the premeiotic stages of mammalian spermatogenesis.[44] *Tex11* is the first X-linked meiosis gene, which forms distinct foci on meiotic chromosomes during male and female gametogenesis. *Tex11*-deficient male mice show defective double-strand break repair and dysregulation of crossing over that further results in apoptosis of spermatocytes at pachytene stage and hence infertility.[45,46] Yatsenko *et al*. had recently identified deletion of three coding exons from *TEX11* gene (99 kb) in two azoospermia men. In addition, they have reported two missense and three splicing mutations in 2.4% of nonobstructive azoospermic men with complete meiotic

arrest.[47] Later, another group identified a different set of *TEX11* mutations in NOA individuals.[48] Thus, *TEX11* seems to be a major X-linked candidate gene for male infertility and is now included in genetic diagnostics of male infertility in Europe. However, there is no study on *TEX11* gene till date in Indian infertile men.

## Genetics of Quantitative Spermatogenic Defects

Quantitative spermatogenic defects due to primary testicular failure can manifest as varied phenotypes ranging from azoospermia (no spermatozoa in the ejaculate) to oligozoospermia (sperm concentration <15 million/ml). Among the different types of quantitative spermatogenic defects, azoospermia is the most severe and common form of male infertility. Men with NOA may present themselves with any of the three testicular histopathologies that include SCOS, mixed testicular atrophy (tubules show varying stages of hypospermatogenesis), and spermatogenic arrest.[49] In men with complete maturation arrest, multiple attempts for testicular sperm extraction for spermatozoa recovery will be futile. Whereas in infertile men with incomplete maturation arrest, round or other later stage spermatids may be seen in the tubules.[49] The etiology of complete or incomplete maturation arrest is not completely understood and genetic factors are expected to play a pivotal role. A total of 13 genes associated with NOA due to maturation/meiotic arrest have been reported by various studies to date.[50-52] However, none of those are currently being used for genetic diagnosis of NOA in routine clinical practice. A list of the genes that have a definitive or strong association with NOA is shown in Table 1.

## Genetics of Qualitative Spermatogenic Defects

Qualitative defects of spermatogenesis identified through routine semen analysis include defects in sperm morphology, motility, and functional parameters such as DNA and chromatin integrity. Various clinical classifications for qualitative defects of spermatogenesis based on semen evaluation include "oligozoospermia" (reduced sperm count), "asthenozoospermia" (reduced sperm motility), and "teratozoospermia" (reduced percentage of sperm with normal morphology). However, other terms such as asthenoteratozoospermia, oligoasthenozoospermia, oligoteratozoospermia, and OAT are also used to describe more than one abnormality in the semen parameters. An interesting syndromic phenotype that gained attention in recent times is multiple morphological abnormalities of the sperm flagella (MMAF), which was first proposed in 2014.[53] MMAF is a type of asthenoteratozoospermia with a mosaic of flagellar morphological defects such as absent, short, bent, coiled, and irregular flagella without systemic ciliary defects such as primary ciliary dyskinesia. Similar phenotypes such as "dysplasia of the fibrous sheath," "short tails," or "stump tails" have been proposed before the term MMAF was proposed.[54-57] However, the term MMAF is now routinely used for asthenozoospermia phenotype after careful assessment of the abnormal morphology of sperm flagella, including the absent, short, bent, coiled, and irregular tail, according to the 5$^{th}$ (2010) and 6$^{th}$ (2021) editions of the World Health Organization standards for the evaluation of human semen. Furthermore, MMAF is not only a mosaic of morphological abnormalities (absent, bent, coiled, short, and irregular tail) but also a mosaic of ultrastructural flagellar defects such as absent central pair, dysplasia of fibrous sheath, disorganised double microtubules, or absence of dynein arms, suggesting the genetic heterogeneity of MMAF phenotype. The list of genes that cause MMAF phenotype identified to date is shown in Table 2.

## Genetics of Obstructive Azoospermia Due to Vas Aplasia

Although the congenital absence of vas deferens (CAVD) was first described in 1755 by Hunter (1786), it took a very long time for recognizing the CAVD as a clinical entity responsible for male infertility. From the mid-twentieth century, urologists started considering CAVD as a separate clinical entity responsible for male infertility.[58] There is a phenotypic diversity in CAVD and at least five phenotypes are known to be associated with male infertility: (1) congenital bilateral absence of the vas deferens (CBAVD) with normal kidneys, (2) CBAVD with unilateral renal anomalies (CBAVD-URA), (3) congenital unilateral absence of the vas deferens (CUAVD), (4) CUAVD-URA, and (5) CBAVD/CUAVD with ejaculatory duct obstruction. Of all these phenotypes, CBAVD is reported in 1%–2% of infertile men.[59] CUAVD is reported in 0.5%–1.0% of men usually discovered during evaluations for infertility or surgical procedures of the male genitalia.[60] CUAVD could be underestimated due to the normal function of the other vas deferens.

Since the CAVD may or may not be associated with anomalies of seminal vesicle (SV) and kidney, it is difficult to elucidate the etiopathogenic mechanisms. SV anomalies can be further classified as hypotrophy, atrophy, dilation, or the absence of bilateral or unilateral SV. There is a variation in the frequency of SV anomalies in CBAVD and CUAVD. Bilateral SV anomalies are more commonly

**Table 1: List of major genes implicated in male infertility with either definitive or strong evidence**

| Gene | Cytogenetic band | Phenotype | Core reference (s) |
|---|---|---|---|
| *FANCM* | 14q21.2 | SCOS, OA, NOA | Kasak *et al.* (2018), Yin *et al.* (2019) |
| *Tex11* | Xq13.1 | MA, mixed testicular atrophy | Yatsenko *et al.* (2015), Yang *et al.* (2015), Nakamura *et al.* (2017), Sha *et al.* (2018) |
| *TEX14* | 17q22 | MA, SCOS resulting in NOA | Fakhro *et al.* (2018), Gershoni *et al.* (2017), An *et al.* (2021) |
| *SYCP2* | 20q13.33 | Oligozoospermia, cryptozoospermia, azoospermia | Schilit SLP *et al.* (2020) |
| *M1AP* | 2p13.1 | Meiotic arrest resulting in NOA | Wyrwoll *et al.* (2020) |
| *STAG3* | 7q22.1 | Meiotic arrest resulting in NOA | van der Bijl *et al.* (2019), Jaillard *et al.* (2020) |
| *MEIOB* | 16p13.3 | SCA resulting in NOA | |
| *SYCE1* | 10q26.3 | SCA resulting in NOA | Pashaei, M *et al.* (2020), Maor-Sagie, E *et al.* (2015) |
| *MEI1* | 22q13.2 | SCA resulting in NOA | Sato, H *et al.* (2006), Ben Khelifa *et al.* (2018), Nguyen *et al.* (2018) |
| *WT1* | 11p13 | SCOC, MA resulting in NOA | Xu *et al.* (2017), Seabra *et al.* (2015), Wang *et al.* (2013) |
| *AURKC* | 19p13.3 | Macrozoospermia, polyploid spermatozoa, teratozoospermia | Hamza *et al.* (2020), Wellard *et al.* (2020), Ben Khelifa *et al.* (2011), Dieterich *et al.* (2007) |
| *DPY19L2* | 12q14.2 | Globozoospermia | Harbuz *et al.* (2011), Ghédir *et al.* (2016), Shang *et al.* (2019) |
| *MSH4* | 1p31.1 | SCA resulting in NOA | Tang *et al.* (2020), Krausz *et al.* (2020) |

Genes implicated in congenital hypogonadotropic hypogonadism and genes requiring additional studies to establish the causative link were excluded. SCOS=Sertoli cell only syndrome, OA=Oligoasthenozoospermia, MA=Maturation arrest, SCA=Spermatocytic arrest, NOA; Nonobstructive azoospermia

reported in CBAVD (50%) than CUAVD (25%), whereas unilateral SV anomalies are more common in CUAVD (80%).[61] Since not all CUAVD cases will be investigated and only those with azoospermia would undergo investigation, the real frequency of SV anomalies in CUAVD would always be underreported. In addition, differences in detection methods can also be responsible for the variation in the frequency of SV anomalies.

For more than two decades, the genetics of CAVD remained restricted to the *CFTR* gene. CBAVD has been extensively studied in Caucasians and genotype–phenotype studies demonstrated two groups of *CFTR* mutations. Severe mutations with virtually no functional CFTR protein or inadequate CFTR protein are classified as Class I, II, and III. The other group is called mild mutations with enough residual CFTR activity to sustain pancreatic function. These *CFTR* mutations are classified as class IV, V.[62] One severe and one mild *CFTR* mutation are detected in 88% of CBAVD men and two mild *CFTR* mutations are detected in 12% of CBAVD, but these men never carry two severe *CFTR* mutations.[59] *CFTR* gene mutations are detected in 60%–70% of isolated CBAVD, and 30%–40% of CBAVD cases may have genetic etiology other than *CFTR*.[63,64] F508del is the most commonly reported *CFTR* gene mutation in Caucasian men with CBAVD, whereas IVS-9 c. 1210-12[5] is the most commonly reported *CFTR* variant in non-Caucasian men with CBAVD [Table 3]. Recently, we reported *CFTR* variants in 66.3% of CBAVD cases, and no *CFTR* variants were detected in 33.7% of CBAVD cases. F508del was reported at a lower allelic frequency (8.75%), whereas the IVS-9 c.1210-12[5] variant was reported at a higher frequency (42.5%).[63] We also investigated female carrier status and observed 13 (16.2%) female partners as cystic fibrosis (CF) carriers. The study also demonstrated that 9 (11%) couples had a risk of transmitting mutant *CFTR* allele to the offspring warranting the *CFTR* screening and genetic counseling before undergoing ICSI. The most challenging question for men with CBAVD is the need for complete sequencing of the *CFTR* gene in both the man and his partner, to evaluate the accurate risk of CF in the offspring, as population-specific *CFTR* mutation panel is not available in India.

Earlier, we observed renal anomalies in 9% of Indian men with CAVD. Renal anomalies were comparatively higher (50%) in CUAVD compared to CBAVD (10%). No major *CFTR* gene mutations were detected in Indian men with CBAVD-URA.[65] Therefore, the etiology of CBAVD-URA could be other than the *CFTR* gene. A recent systematic review reported association of IVS-9

**Table 2: List of currently identified genes that are associated with multiple morphological abnormalities of the sperm flagella phenotype**

| Gene | Cytogenetic band | Protein localization in sperm | Sperm phenotype (human) | ICSI outcome (positive outcome/ total number of patients) | Reference |
|---|---|---|---|---|---|
| *DNAH1* | 3p21.2 | IDA | Absent IDA; disorganised 9+2; absent CP; disorganized FS | Pregnancy (5/6) | Ben Khelifa M *et al.* (2014), Pazour *et al.* (1999), Wambergue *et al.* (2016), Sha *et al.* (2017), Liu *et al.* (2019a), Wang *et al.* (2017), Amiri-Yekta *et al.* (2016) |
| *DNAH2* | 17p13.1 | IDA | Absent IDA; disorganised 9+2; absent CP; disorganized FS | Pregnancy (2/2) | Kamiya *et al.* (1991), Li *et al.* (2019c), Hwang *et al.* (2021), Gao *et al.* (2021) |
| *DNAH6* | 2p11.2 | IDA | Absent IDA; disorganised 9+2; absent CP | Abortion (1/1) | Tu *et al.* (2019) |
| *DNAH8* | 6p21.2 | ODA | Absent or disarranged ODA; absent CP; other axonemal anomalies | - | Liu *et al.* (2020), Yang *et al.* (2020), Weng *et al.* (2021) |
| *DNAH17* | 17q25.3 | ODA | Absent or disarranged ODA; absent CP | - | Chaofeng *et al.* (2021) |
| *CFAP43* | 10q25.1 | Near DMT | Lack of CP; FS hyperplasia; short tails with unorganised cytoplasm | Pregnancy (1/1) | Tang *et al.* (2017), Fu *et al.* (2018), Yu *et al.* (2021), sha *et al.* (2019b, c), Coutton *et al.* (2018), Li *et al.* (2020) |
| *CFAP44* | 3q13.2 | Paraflagellar rod | Absent CP; disorganised DMT | Pregnancy (2/4) | Tang *et al.* (2017), Jin *et al.* (2017), Sha *et al.* (2019b, c), Wu *et al.* (2019) |
| *CFAP65* | 2q35 | Localises with T/TH complex | Absent CP; hypertrophy and hyperplasia of FS | Abortion (3/3) | Li *et al*. (2020) |
| *CFAP70* | 10q22.2 | ODA | Absent sperm tail; disorganised ODA | Pregnancy (1/1) | Shomoto *et al.* (2018), Julie *et al.* (2019), Beurois et al (2019) |
| *AK7* | 14q32.2 | Sperm flagella | Dysplasia of FS; lack of CP; incomplete mitochondrial sheath | - | Frenandex-Gonza-Lez *et al.* (2009), Lorès *et al.* (2018) |
| *CFAP251* | 12q24.31 | Sperm flagella | | - | Li *et al.* (2019), Kherraf *et al.* (2018), Heuser *et al.* (2012a, b), Urbanska *et al.* (2015) |
| *FSIP2* | 2q32.1 | FS of flagella | Complete absence of mitochondrial sheath; hypertrophic FS | - | Guillaume *et al.* (2018), Brown *et al.* (2003) |
| *AKAP4* | Xp11.22 | Outer dense fibre for sperm | High XY disomy; disorganised FS; altered axonemal structure; FS remnants embedded in a cytoplasmic residue | - | Baccett *et al.* (2005) |
| *QRICH2* | 17q25.1 | Sperm flagella | Absent CP, disorganised ODF, Absent FS | - | Shen *et al.* (2019) |

ICSI=Intracytoplasmic sperm injection, CP=Central pair, DMT=Double microtubule, ODA=Outer dynein arm, IDA=Inner dynein arm, ODF=Outer dense fibre, FS=Fibrous sheath

c.1210-12[5] with increased risk of CUAVD, and renal anomalies are more common in CUAVD than CBAVD. However, renal anomalies in CBAVD or CUAVD were not associated with *CFTR* variants.[66]

With the availability of NGS, attempts are ongoing to unravel the genetic etiology other than *CFTR*. A new pathogenic gene *ADGRG2* was detected in 11%–15% of *CFTR*-negative CBAVD.[67,68] *PANK2*, *SCNN1B*, and *CA12* are also reported in CBAVD.[69,70] These shreds of evidence suggest the need for expanding diagnostics of CAVD in addition to *CFTR*.

## Male Infertility Genetics: Challenges and Future

As described earlier, human spermatogenesis is highly complex and is driven by the regulated expression of

**Table 3: Cystic fibrosis transmembrane conductance regulator gene mutations in Indian men with congenital bilateral absence of vas deferens**

| Study population and phenotype studied (sample size) | F508del (%) | IVS-9 c. 1210-12[5] 5T allele (%) | Novel CFTR gene mutations | No CFTR gene mutations (%) | Reference |
|---|---|---|---|---|---|
| North Indian CBAVD (*n*=40) CUAVD (*n*=10) | 11 | 25 | L69H, F87I, G126S, F157C, E543A, Y852F, D1270E | 26% | Sharma *et al.*, 2009 |
| North Indian CBAVD (*n*=35) | 17.1 | 27.1 | E217Gfs*11, A1285V | - | Sachdeva *et al.*, 2011 |
| Pan-India CBAVD (*n*=80) | 8.75 | 42.5 | L214V, A238P, E379V, L578I, F587L, L926W, R1325K R1453Q c. 1-30C>G IVS1+2T>G | 33.7 | Gaikwad *et al.*, 2020 |

CBAVD=Congenital bilateral absence of vas deferens, CUAVD=Congenital unilateral absence of vas deferens, CFTR=Cystic fibrosis transmembrane conductance regulator

many genes. Therefore, the observed phenotypes in male infertility after semen evaluation are rather a clinical endpoint of a spectrum of alternative pathological processes.[4] The observed male infertility phenotypes may manifest themselves through *de novo* variants or autosomal recessive pathogenic variants inherited from fertile parents. In the routine diagnosis of male infertility, genetic testing is currently being used for identifying chromosomal anomalies, Y chromosome microdeletions, and pathogenic variants linked with congenital hypogonadotropic hypogonadism. Indeed, no new genetic causes that impact clinical diagnostic workup or treatment decisions have been identified in over 20 years.[11,71,72] Currently, no population-specific genetic markers for oligozoospermia, NOA, and obstructive azoospermia due to vas aplasia are available in India.

With the advancement in genomic technologies, it is now possible to sequence the whole exome or specific genes of interest (targeted gene resequencing) and identify multiple autosomal pathogenic variants in infertile men. Genome-wide association analysis is another approach that was used in recent times to identify susceptible loci linked with male infertility. In India, there is an unmet need of investigating the genes implicated in male infertility in other populations. However, it should be noted that some of the genes such as *NR5A1* that were reported to be associated with male infertility in other populations are not associated with male infertility in Indian men.[73] Therefore, there is a need to rule out the association of genes reported in other populations in Indian infertile men. Further, to identify novel genes with diagnostic value, a stringent and careful evaluation of male infertility phenotypes is important. Wherever possible, recruitment of familial cases, testicular histopathology (in cases of NOA), and electron microscope imaging of spermatozoa (in case of suspected MMAF phenotypes) are extremely useful. As mentioned earlier, our data suggest that a large proportion (33.7%) of Indian men with CBAVD may have non-*CFTR* genetic causes, and in such cases, whole-exome sequencing would be useful to identify novel genes.

## Conclusion

The review demonstrates a need for careful evaluation of the male infertility phenotype, clinical classification, and usage of advanced genomic technologies to uncover monogenic causes of male infertility with diagnostic implications. The genomic analysis needs to be quickly implemented in clinical practice as the detection of gene abnormalities will facilitate genetic counseling with the patients. The genetic testing guidelines of male infertility need to be regularly updated based on the evidence from genomic analysis data. National agencies should be involved in the development of population-specific genetic testing panels.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, *et al.* Male infertility. Lancet 2021;397:319-33.
2. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. PLoS Med 2012;9:e1001356.
3. Neto FT, Bach PV, Najar i BB, Li PS, Goldstein M. Genetics of

male infertility. Curr Urol Rep 2016;17:70.
4. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol 2018;15:369-84.
5. Tournaye H, Krausz C, Oates RD. Novel concepts in the aetiology of male reproductive impairment. Lancet Diabetes Endocrinol 2017;5:544-53.
6. Krausz C. Male infertility: Pathogenesis and clinical diagnosis. Best Pract Res Clin Endocrinol Metab 2011;25:271-85.
7. Vincent MC, Daudin M, De MP, Massat G, Mieusset R, Pontonnier F, *et al.* Cytogenetic investigations of infertile men with low sperm counts: A 25-year experience. J Androl 2002;23:18-22.
8. Palermo GD, Colombero LT, Hariprashad JJ, Schlegel PN, Rosenwaks Z. Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod 2002;17:570-5.
9. Walsh TJ, Pera RR, Turek PJ. The genetics of male infertility. Semin Reprod Med 2009;27:124-36.
10. Punab M, Poolamets O, Paju P, Vihljajev V, Pomm K, Ladva R, *et al.* Causes of male infertility: A 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod 2017;32:18-31.
11. McLachlan RI, O'Bryan MK. Clinical review#: State of the art for genetic testing of infertile men. J Clin Endocrinol Metab 2010;95:1013-24.
12. Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, *et al.* Cytogenetics of infertile men. Hum Reprod 1996;11 Suppl 4:1-24.
13. Engels H, Eggermann T, Caliebe A, Jelska A, Schubert R, Schüler HM, *et al.* Genetic counseling in Robertsonian translocations der (13;14): Frequencies of reproductive outcomes and infertility in 101 pedigrees. Am J Med Genet A 2008;146A: 2611-6.
14. Mozdarani H, Meybodi AM, Karimi H. Impact of pericentric inversion of Chromosome 9 [inv (9) (p11q12)] on infertility. Indian J Hum Genet 2007;13:26-9.
15. Sismani C, Rapti SM, Iliopoulou P, Spring A, Neroutsou R, Lagou M, *et al.* Novel pericentric inversion inv (9)(p23q22.3) in unrelated individuals with fertility problems in the Southeast European population. J Hum Genet 2020;65:783-95.
16. Ali S, Hasnain SE. Genomics of the human Y-chromosome. 1. Association with male infertility. Gene 2003;321:25-37.
17. Tilford CA, Kuroda-Kawaguchi T, Skaletsky H, Rozen S, Brown LG, Rosenberg M, *et al*. A physical map of the human Y chromosome. Nature 2001;409:943-5.
18. Rozen S, Skaletsky H, Marszalek JD, Minx PJ, Cordum HS, Waterston RH, *et al.* Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. Nature 2003;423:873-6.
19. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, *et al.* The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003;423:825-37.
20. Lahn BT, Page DC. Functional coherence of the human Y chromosome. Science 1997;278:675-80.
21. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 1976;34:119-24.
22. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, *et al.* Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet 1996;5:933-43.
23. Yu XW, Wei ZT, Jiang YT, Zhang SL. Y chromosome azoospermia factor region microdeletions and transmission characteristics in azoospermic and severe oligozoospermic patients. Int J Clin Exp Med 2015;8:14634-46.
24. Colaco S, Modi D. Genetics of the human Y chromosome and its association with male infertility. Reprod Biol Endocrinol 2018;16:14.
25. Krausz C, Casamonti E. Spermatogenic failure and the Y chromosome. Hum Genet 2017;136:637-55.
26. Punjani N, Kang C, Schlegel PN. Clinical implications of Y chromosome microdeletions among infertile men. Best Pract Res Clin Endocrinol Metab 2020;34:101471.
27. Bansal SK, Jaiswal D, Gupta N, Singh K, Dada R, Sankhwar SN, *et al.* Gr/gr deletions on Y-chromosome correlate with male infertility: An original study, meta-analyses, and trial sequential analyses. Sci Rep 2016;6:19798.
28. Liu XG, Hu HY, Guo YH, Sun YP. Correlation between Y chromosome microdeletion and male infertility. Genet Mol Res 2016;15:gmr8426.
29. Thangaraj K, Gupta NJ, Pavani K, Reddy AG, Subramainan S, Rani DS, *et al*. Y chromosome deletions in azoospermic men in India. J Androl 2003;24:588-97.
30. Rani DS, Rajender S, Pavani K, Chaubey G, Rasalkar AA, Gupta NJ, *et al.* High frequencies of Non Allelic Homologous Recombination (NAHR) events at the AZF loci and male infertility risk in Indian men. Sci Rep 2019;9:6276.
31. Maurer B, Simoni M. Y chromosome microdeletion screening in infertile men. J Endocrinol Invest 2000;23:664-70.
32. Krausz C, Chianese C. Genetic testing and counselling for male infertility. Curr Opin Endocrinol Diabetes Obes 2014;21:244-50.
33. Kuroda-Kawaguchi T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, *et al.* The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 2001;29:279-86.
34. Repping S, Skaletsky H, Lange J, Silber S, Van Der Veen F, Oates RD, *et al.* Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. Am J Hum Genet 2002;71:906-22.
35. Repping S, van Daalen SK, Korver CM, Brown LG, Marszalek JD, Gianotten J, *et al*. A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. Genomics 2004;83:1046-52.
36. Navarro-Costa P, Gonçalves J, Plancha CE. The AZFc region of the Y chromosome: At the crossroads between genetic diversity and male infertility. Hum Reprod Update 2010;16:525-42.
37. Repping S, Skaletsky H, Brown L, van Daalen SK, Korver CM, Pyntikova T, *et al.* Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet 2003;35:247-51.
38. Shahid M, Dhillon VS, Khalil HS, Sexana A, Husain SA. Associations of Y-chromosome subdeletion gr/gr with the prevalence of Y-chromosome haplogroups in infertile patients. Eur J Hum Genet 2011;19:23-9.
39. Visser L, Westerveld GH, Korver CM, van Daalen SK, Hovingh SE, Rozen S, *et al*. Y chromosome gr/gr deletions are a risk factor for low semen quality. Hum Reprod 2009;24:2667-73.
40. Stahl PJ, Mielnik A, Margreiter M, Marean MB, Schlegel PN, Paduch DA. Diagnosis of the gr/gr Y chromosome microdeletion does not help in the treatment of infertile American men. J Urol 2011;185:233-7.
41. Wu B, Lu NX, Xia YK, Gu AH, Lu CC, Wang W, *et al*. A frequent Y chromosome b2/b3 subdeletion shows strong association with male infertility in Han-Chinese population. Hum

Reprod 2007;22:1107-13.
42. Zhang F, Lu C, Li Z, Xie P, Xia Y, Zhu X, *et al.* Partial deletions are associated with an increased risk of complete deletion in AZFc: A new insight into the role of partial AZFc deletions in male infertility. J Med Genet 2007;44:437-44.
43. Zheng K, Yang F, Wang PJ. Regulation of male fertility by X-linked genes. J Androl 2010;31:79-85.
44. Wang PJ, McCarrey JR, Yang F, Page DC. An abundance of X-linked genes expressed in spermatogonia. Nat Genet 2001;27:422-6.
45. Adelman CA, Petrini JH. ZIP4H (TEX11) deficiency in the mouse impairs meiotic double strand break repair and the regulation of crossing over. PLoS Genet 2008;4:e1000042.
46. Yang F, Gell K, van der Heijden GW, Eckardt S, Leu NA, Page DC, *et al.* Meiotic failure in male mice lacking an X-linked factor. Genes Dev 2008;22:682-91.
47. Yatsenko AN, Georgiadis AP, Röpke A, Berman AJ, Jaffe T, Olszewska M, *et al.* X-linked TEX11 mutations, meiotic arrest, and azoospermia in infertile men. N Engl J Med 2015;372:2097-107.
48. Yang F, Silber S, Leu NA, Oates RD, Marszalek JD, Skaletsky H, *et al.* TEX11 is mutated in infertile men with azoospermia and regulates genome-wide recombination rates in mouse. EMBO Mol Med 2015;7:1198-210.
49. Tüttelmann F, Ruckert C, Röpke A. Disorders of spermatogenesis: Perspectives for novel genetic diagnostics after 20 years of unchanged routine. Med Genet 2018;30:12-20.
50. Riera-Escamilla A, Enguita-Marruedo A, Moreno-Mendoza D, Chianese C, Sleddens-Linkels E, Contini E, *et al.* Sequencing of a 'mouse azoospermia' gene panel in azoospermic men: Identification of RNF212 and STAG3 mutations as novel genetic causes of meiotic arrest. Hum Reprod 2019;34:978-88.
51. Schilit SL, Menon S, Friedrich C, Kammin T, Wilch E, Hanscom C, *et al.* SYCP2 translocation-mediated dysregulation and frameshift variants cause human male infertility. Am J Hum Genet 2020;106:41-57.
52. van der Bijl N, Röpke A, Biswas U, Wöste M, Jessberger R, Kliesch S, *et al.* Mutations in the stromal antigen 3 (STAG3) gene cause male infertility due to meiotic arrest. Hum Reprod 2019;34:2112-9.
53. Ben Khelifa M, Coutton C, Zouari R, Karaouzène T, Rendu J, Bidart M, *et al.* Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. Am J Hum Genet 2014;94:95-104.
54. Moretti E, Geminiani M, Terzuoli G, Renieri T, Pascarelli N, Collodel G. Two cases of sperm immotility: A mosaic of flagellar alterations related to dysplasia of the fibrous sheath and abnormalities of head-neck attachment. Fertil Steril 2011;95:1787.e19-23.
55. Neugebauer DC, Neuwinger J, Jockenhövel F, Nieschlag E. '9+0' axoneme in spermatozoa and some nasal cilia of a patient with totally immotile spermatozoa associated with thickened sheath and short midpiece. Hum Reprod 1990;5:981-6.
56. Olmedo SB, Rawe VY, Nodar FN, Galaverna GD, Acosta AA, Chemes HE. Pregnancies established through intracytoplasmic sperm injection (ICSI) using spermatozoa with dysplasia of fibrous sheath. Asian J Androl 2000;2:125-30.
57. Stalf T, Sánchez R, Köhn FM, Schalles U, Kleinstein J, Hinz V, *et al.* Pregnancy and birth after intracytoplasmic sperm injection with spermatozoa from a patient with tail stump syndrome. Hum Reprod 1995;10:2112-4.
58. Nelson RE. Congenital absence of the vas deferens; a review of the literature and report of three cases. J Urol 1950;63:176-82.
59. Hussein TM, Zakaria NH, Zahran AM. Clinical, laboratory and genetic assessment of patients with congenital bilateral absent vas deferens. Andrologia 2011;43:16-22.
60. Weiske WH, Sälzler N, Schroeder-Printzen I, Weidner W. Clinical findings in congenital absence of the vasa deferentia. Andrologia 2000;32:13-8.
61. Schlegel PN, Shin D, Goldstein M. Urogenital anomalies in men with congenital absence of the vas deferens. J Urol 1996;155:1644-8.
62. Boyle MP, De Boeck K. A new era in the treatment of cystic fibrosis: Correction of the underlying CFTR defect. Lancet Respir Med 2013;1:158-63.
63. Gaikwad A, Khan S, Kadam S, Shah R, Kulkarni V, Kumaraswamy R, *et al.* Cystic fibrosis transmembrane conductance regulator-related male infertility: Relevance of genetic testing and counselling in Indian population. Indian J Med Res 2020;152:575-83.
64. Lu S, Cui Y, Li X, Zhang H, Liu J, Kong B, *et al.* Association of cystic fibrosis transmembrane-conductance regulator gene mutation with negative outcome of intracytoplasmic sperm injection pregnancy in cases of congenital bilateral absence of vas deferens. Fertil Steril 2014;101:1255-60.
65. Gajbhiye R, Kadam K, Khole A, Gaikwad A, Kadam S, Shah R, *et al.* Cystic fibrosis transmembrane conductance regulator (CFTR) gene abnormalities in Indian males with congenital bilateral absence of vas deferens and renal anomalies. Indian J Med Res 2016;143:616-23.
66. Millson A, Pont-Kingdon G, Page S, Lyon E. Direct molecular haplotyping of the IVS-8 poly (TG) and polyT repeat tracts in the cystic fibrosis gene by melting curve analysis of hybridization probes. Clin Chem 2005;51:1619-23.
67. Patat O, Pagin A, Siegfried A, Mitchell V, Chassaing N, Faguer S, *et al.* Truncating mutations in the adhesion G protein-coupled receptor G2 gene ADGRG2 cause an X-linked congenital bilateral absence of vas deferens. Am J Hum Genet 2016;99:437-42.
68. Yang B, Wang J, Zhang W, Pan H, Li T, Liu B, *et al.* Pathogenic role of ADGRG2 in CBAVD patients replicated in Chinese population. Andrology 2017;5:954-7.
69. Shen Y, Yue HX, Li FP, Hu FY, Li XL, Wan Q, *et al.* SCNN1B and CA12 play vital roles in occurrence of congenital bilateral absence of vas deferens (CBAVD). Asian J Androl 2019;21:525-7.
70. Wu YN, Chen KC, Wu CC, Lin YH, Chiang HS. SLC9A3 affects vas deferens development and associates with Taiwanese congenital bilateral absence of the vas deferens. Biomed Res Int 2019;2019:3562719.
71. Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: Role of genetic background. Reprod Biomed Online 2007;14:734-45.
72. Nuti F, Krausz C. Gene polymorphisms/mutations relevant to abnormal spermatogenesis. Reprod Biomed Online 2008;16:504-13.
73. Sudhakar DV, Nizamuddin S, Manisha G, Devi JR, Gupta NJ, Chakravarthy BN, *et al.* NR5A1 mutations are not associated with male infertility in Indian men. Andrologia 2018;50:e12931.