

## R E V I E W

## Non-syndromic monogenic male infertility

*Giulia Guerri<sup>1</sup>, Tiziana Maniscalchi<sup>2</sup>, Shila Baratt<sup>2</sup>, Gian Maria Busetto<sup>3</sup>,  
Francesco Del Giudice<sup>3</sup>, Ettore De Berardinis<sup>3</sup>, Rossella Cannarella<sup>4</sup>, Aldo Eugenio Calogero<sup>4</sup>,  
Matteo Bertelli<sup>2</sup>*

<sup>1</sup>MAGI's Lab, Rovereto (TN), Italy; <sup>2</sup>MAGI Euregio, Bolzano, Italy; <sup>3</sup>Department of Urology, University of Rome La Sapienza, Policlinico Umberto I, Rome, Italy; <sup>4</sup>Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

**Summary.** Infertility is a widespread clinical problem affecting 8-12% of couples worldwide. Of these, about 30% are diagnosed with idiopathic infertility since no causative factor is found. Overall 40-50% of cases are due to male reproductive defects. Numerical or structural chromosome abnormalities have long been associated with male infertility. Monogenic mutations have only recently been addressed in the pathogenesis of this condition. Mutations of specific genes involved in meiosis, mitosis or spermiogenesis result in spermatogenic failure, leading to the following anomalies: insufficient (oligozoospermia) or no (azoospermia) sperm production, limited progressive and/or total sperm motility (asthenozoospermia), altered sperm morphology (teratozoospermia), or combinations thereof. Androgen insensitivity, causing hormonal and sexual impairment in males with normal karyotype, also affects male fertility. The genetic causes of non-syndromic monogenic of male infertility are summarized in this article and a gene panel is proposed. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** male infertility, oligozoospermia, azoospermia, asthenozoospermia, teratozoospermia, spermatogenic failure, androgen insensitivity syndrome

### Introduction

Infertility is defined as failure to achieve a successful pregnancy after 12 months or more of regular unprotected intercourse (1). Its prevalence is not negligible, since about 48.5 million couples worldwide do not reach pregnancy after 5 years (2). Overall, about 50% of cases are due to a male factor infertility (3). Genetic causes have been estimated to exist in about 15% of infertile patients, especially in those with azoospermia or severe oligozoospermia (3).

Genetic causes of male infertility can be classified as pre-testicular (affecting hypothalamic-pituitary function), testicular (causing dysfunction at testicular level) and post-testicular (leading to obstruction or interfering with ejaculation of sperm). Other causes include androgen resistance and disorders of sexual

development. Genetic causes of male infertility are outlined in Table 1.

Despite a proper diagnostic work-up, the etiology of male infertility remains elusive in up to 75% of cases (4). In recent years, much effort has been made to investigate new candidate genes responsible for male infertility caused by single-gene mutations (5,6). Several genes involved in meiotic and mitotic divisions and in spermiogenesis have been examined as potential targets. They may play a role in the pathogenesis of defects of sperm number (oligozoospermia or azoospermia), motility (asthenozoospermia) or morphology (teratozoospermia) (7).

In this review we describe genes belonging to the panels developed by us for the diagnosis of monogenic spermatogenic failure and androgen insensitivity syndrome.

**Table 1.** Main causes of genetic forms of male infertility.

Pre-testicular causes	<ul style="list-style-type: none"> <li>• Normosmic hypogonadotropic hypogonadism</li> <li>• Anosmic hypogonadotropic hypogonadism (Kallmann syndrome)</li> <li>• Prader-Willy syndrome</li> <li>• Laurence-Moon-Biedl syndrome</li> <li>• Others</li> </ul>
Testicular forms	<ul style="list-style-type: none"> <li>• Klinefelter syndrome</li> <li>• Numerical chromosomal abnormalities</li> <li>• Y chromosome microdeletions</li> <li>• Chromosomal translocations</li> <li>• Down syndrome</li> <li>• Myotonic dystrophy (Steinert syndrome)</li> </ul>
Post-testicular causes	<ul style="list-style-type: none"> <li>• Kartagener syndrome</li> <li>• Congenital bilateral deferent duct agenesis</li> <li>• Young syndrome</li> </ul>
Others	<ul style="list-style-type: none"> <li>• Androgen resistance</li> <li>• Disorders of sexual development</li> </ul>

### Genes involved in sperm number defects

Sperm number defects include azoospermia and oligozoospermia. Azoospermia is the absence of spermatozoa in semen. It affects 1% of the male population and accounts for 20% of all cases of male infertility (8). In about the 40% of patients, spermatogenesis occurs in a regular way but sperm emission is impaired by seminal duct obstruction (obstructive azoospermia) (9); in the other cases, azoospermia is due to spermatogenic failure (non-obstructive azoospermia) (10). Genetic causes of azoospermia include chromosome anomalies (numerical or structural aberrations of autosomal or sexual chromosomes) that affect 5% of all infertile males and 16% of males with azoospermia or oligozoospermia (11). In 5-15% of cases, azoospermia or oligozoospermia is associated with Y chromosome microdeletions; 6-8% of cases with obstructive azoospermia are associated with mutations in the cystic fibrosis transmembrane receptor gene (*CFTR*) that causes congenital bilateral absence of the *vas deferens* (11). Point mutations that cause azoospermia were recently found in the following genes: *NR5A1*, *SYCP3*, *ZMYND15*, *TAF4B*, *TEX11*, *NANOS1*, *PLK4*, *MEIOB*, *SYCE1*, *USP9Y*, *SOHLH1*, *TEX15*, *HSF2* and *KLHL10* (12-19) (Table 2).

Frameshift mutations in *ZMYND15* cause the SPGF14 phenotype. The protein encoded by this gene

is involved in temporally normal haploid gene expression during spermatogenesis (13).

A homozygous mutation in *SYCE1* is associated with the SPGF15 phenotype. This gene encodes a member of the synaptonemal complex, a structure that physically links homologous chromosomes during meiosis I (14).

Mutations in *TEX11* have been associated with meiotic arrest and azoospermia with a frequency of 1-15% in the azoospermic males. *TEX11*-encoded protein regulates the coupling of homologous chromosomes in double-strand DNA repair through formation of the synaptonemal complex and the chiasma during the crossover process (20). A similar role is performed by *SYCP3* that has also been found mutated in sterile men (16).

*SOHLH1* is mutated in some cases of azoospermia and encodes a testicular transcription factor essential for spermatogenesis (21).

A mutation in *NR5A1*, encoding steroidogenic factor 1, has been reported in a Pakistani patient with meiotic arrest and normal levels of follicle-stimulating and luteinizing hormones, and testosterone (22).

Finally, a patient with spermatogenesis blocked at the spermatocyte stage had a dominant negative mutation in *HSF2*, encoding heat shock transcription factor 2 (23).

**Table 2.** Genes associated with spermatogenic failure

Gene	Inheritance	OMIM gene	OMIM phenotype	OMIM or HGMD phenotype ID	Spermatogenic defect
<i>NR5A1</i>	AR	184757	SPGF8	613957	AZS/OZS
<i>SYCP3</i>	AD	604759	SPGF4	270960	AZS/OZS
<i>ZMYND15</i>	AR	614312	SPGF14	615842	AZS/OZS
<i>TAF4B</i>	AR	601689	SPGF13	615841	AZS/OZS
<i>TEX11</i>	XLR	300311	SPGFX2	309120	AZS
<i>NANOS1</i>	AD	608226	SPGF12	615413	AZS/OZS/OZS+ASTHZ+TZS
<i>PLK4</i>	AD	605031	/	1556988045	AZS
<i>MEIOB</i>	AR	617670	SPGF22	617706	AZS
<i>SYCE1</i>	AR	611486	SPGF15	616950	AZS
<i>USP9Y</i>	YL	400005	SPGFY2	400042	AZS
<i>SOHLH1</i>	AD	610224	SPGF32	618115	AZS
<i>TEX15</i>	AR	605795	SPGF25	617960	AZS/OZS
<i>HSF2</i>	AD	140581	/	702994563	AZS
<i>KLHL10</i>	AD	608778	SPGF11	615081	OZS; TZS; AZS
<i>AURKC</i>	AR	603495	SPGF5	243060	TZS (macrozoospermia)
<i>DPY19L2</i>	AR	613893	SPGF9	613958	TZS (globozoospermia)
<i>SPATA16</i>	AR	609856	SPGF6	102530	TZS (globozoospermia)
<i>PICK1</i>	AR	605926	/	247048065	TZS (globozoospermia)
<i>BRDT</i>	AR	602144	SPGF21	617644	ASS
<i>SUN5</i>	AR	613942	SPGF16	617187	ASS
<i>SLC26A8</i>	AD	608480	SPGF3	606766	AZS
<i>CATSPER1</i>	AR	606389	SPGF7	612997	AZS
<i>SEPT12</i>	AD	611562	SPGF10	614822	AZS; OZS+ASTHZ+TZS
<i>CFAP43</i>	AR	617558	SPGF19	617592	MMAF
<i>CFAP44</i>	AR	617559	SPGF20	617593	MMAF
<i>DNAH1</i>	AR	603332	SPGF18	617576	MMAF
<i>PLCZ1</i>	AR	608075	SPGF17	617214	OAF

SPGF = spermatogenic failure; OZS = oligozoospermia; AZS = azoospermia; ASTHZ = asthenozoospermia; TZS = teratozoospermia; OZS+ASTHZ+TZS = oligoasthenoteratozoospermia; ASS = acephalic spermatozoa syndrome; MMAF = multiple morphological abnormalities of the flagellum; OAF = oocyte activation failure; AR = autosomal recessive; AD = autosomal dominant; XLR = X-linked recessive; YL = Y-linked; HGMD = Human Gene Mutation Database (<https://portal.biobase-international.com/hgmd/pro/>)

## Genes involved in defects of sperm morphology

Teratozoospermia is a heterogeneous group of disorders. Sperm morphological evaluation considers the main functional regions (head, body and tail), which may have anomalies in shape and size. Phenotype may involve a single type of malformation in a single patient or different types of malformation in the same patient (24). Recent studies in consanguineous families and small phenotypically homogeneous cohorts has made it possible to identify autosomal recessive cases of teratozoospermia.

Macrozoospermia is a rare condition observed in <1% of infertile men. It is characterized by a high percentage of sperm with large irregularly-shaped heads, multiple flagella and abnormal acrosome. Macrozoospermia is generally associated with severe oligoasthenozoospermia and a high rate of sperm chromosomal anomalies. Homozygous mutations in *AURKC* are the major cause of macrozoospermia. *AURKC* is highly expressed in male germline cells and is involved in chromosomal segregation and cytokinesis (25).

Globozoospermia is a rare condition characterized by round sperm lacking acrosomes. Genes reported mutated in patients with this condition are *SPATA16* and *DPY19L2*. *SPATA16* encodes a protein specific to Golgi apparatus, highly expressed in the testes. A genetic study in a single consanguineous family with male infertility due to globozoospermia revealed a homozygous variant in the three affected siblings (26). Subsequent studies identified mutations in *DPY19L2* in 66.7% of 54 probands with globozoospermia (25). The protein encoded by *DPY19L2* is necessary for elongation of the sperm head and acrosome formation during spermatogenesis (27).

To date, more than 20 cases of patients with acéphalic sperm have been reported. Biallelic mutations in *SUN5* can be found in 47% of cases (28), and a homozygous mutation in *BRDT* has been reported in a consanguineous family (27).

Genes currently associated with morphological sperm defects are: *AURKC*, *ZPBP*, *DPY19L2*, *SPATA16*, *PICK1*, *BRDT* and *SUN5* (Table 2).

## Genes involved in sperm motility defects

Asthenozoospermia is a condition leading to reduced sperm motility due to defects in the flagellum. The axoneme, outer dense fibers, mitochondria or fibrous sheath of the flagellum may be affected (30). Ultrastructural defects in 9+2 axoneme structure may involve the outer or inner dynein arms, central microtubules and radial spokes.

Biallelic mutations in *DNAH1*, that encodes heavy chain 1 of axonemal dynein expressed in the testis, cause a heterogeneous group of anomalies defined as multiple morphological anomalies of the flagellum (MMAF) (31,32). Mutations in *DNAH1* are the major cause of MMAF and account for 28-44% of cases (33). In four out of 30 Chinese subjects with MMAF, Tang et al. identified mutations in either *CFAP43* or *CFAP44*. The proteins encoded, *CFAP43* and *CFAP44*, are cilium- and flagellum-associated, almost exclusively expressed in the testis.

The genes associated with sperm motility defects are: *SLC26A8*, *CATSPER1*, *SEPT12*, *CFAP43*, *CFAP44*, *DNAH1* and *PLCZ1* (Table 2).

We use a multi-gene NGS panel to detect nucleotide variations in coding exons and flanking introns of the aforementioned genes (Table 2). Pathogenic variants may be missense, nonsense, splicing or small indels. Large deletions/duplications have been reported in *USP9Y*, *DPY19L2*, *SPATA16*, *SUN5* and *CFAP43*. We use MLPA to detect duplications and deletions in the latter genes.

## Androgen insensitivity syndrome

Androgens and their receptor are essential for the development and maintenance of the male phenotype and spermatogenesis. The gene *AR* encoding the androgen receptor is X-linked and the encoded protein is a nuclear receptor that recognizes the canonical androgen response elements on the genome (34).

Mutations in *AR* (OMIM gene ID: 313700) cause a spectrum of conditions defined as androgen insensitivity syndromes (OMIM phenotype ID: 300068), which are disorders of sexual development characterized by external female genitalia, ambiguous genitalia or virilization defects, 46,XY karyotype and

little or no response to androgens. Androgen insensitivity syndromes (AIS) may be complete or partial. Patients with the complete form have a female phenotype with little or no pubic/axillary hair or secondary sexual features and a 46,XY karyotype (35).

Mutations can be found throughout the gene, but are more frequent in five exons that encode ligand-binding domains. The androgen insensitivity syndrome phenotype is due to loss-of-function mutations in *AR*, making target cells insensitive to testosterone or dihydrotestosterone. In 95% of cases, a mutation in the *AR* gene can be detected. In 30% of cases the mutations are *de novo*. The disorder is inherited with a X-linked recessive inheritance (36).

Clinical diagnosis is based on symptoms and biochemical features of 46,XY females. The typical hormonal profile of adults includes increased basal concentrations of luteinizing hormone and testosterone. Serum levels of anti-Müllerian hormone may be normal or elevated. Subjects with partial loss-of-function mutations in *AR* (partial AIS) can have infertility as first or only symptom. Partial androgen insensitivity syndrome may be suspected in infertile males with high plasma levels of testosterone and LH, although a precise threshold has not yet been established (36).

Interestingly, mutations in *AR* can be identified in 2-3% of cases of azoospermia and oligozoospermia (38). There may be a genotype-phenotype correlation in AIS patients, as reported in the Androgen Receptor Gene Mutations Database (39), whereas a correlation in partial AIS patients is less clear and the same mutations can express as different phenotypes (37). The *AR* gene may therefore be included among candidate genes in patients with apparently idiopathic azoospermia or oligozoospermia.

## Conclusions

We created a NGS panel to detect nucleotide variations in coding exons and flanking regions of all the genes associated with infertility. When a suspect of male infertility is present we perform the analysis of all the genes present in this short article.

In order to have a high diagnostic yield, we developed a NGS test that reaches an analytical sensitivity (proportion of true positives) and an analytical speci-

ficity (proportion of true negatives) of  $\geq 99\%$  (coverage depth  $\geq 10x$ ).

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Correspondence:  
Stefano Paolacci  
Via delle Maioliche 57/D - 38068 Rovereto (TN), Italy  
E-mail: stefano.paolacci@assomagi.org