Chapter 16
Monogenic Forms of Male Infertility

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Abstract Male infertility is a multifactorial and heterogeneous pathological condition affecting 7% of the general male population. The genetic landscape of male infertility is highly complex as semen and testis histological phenotypes are extremely heterogeneous, and at least 2000 genes are predicted to be involved in spermatogenesis. Genetic factors have been described in each etiological category of male reproductive impairment: (1) hypothalamic–pituitary axis dysfunction; (2) quantitative and qualitative alterations of spermatogenesis; (3) ductal obstruction/dysfunction. In 25% of azoospermic and in 10% of oligozoospermic men, a genetic anomaly can be diagnosed with the current genetic testing. However, up to now, only a relatively low number of monogenic factors have a clear-cut cause–effect relationship with impaired reproductive function. Thanks to the widespread diffusion of Next-Generation Sequencing, a continuously increasing number of monogenic causes of male infertility are being discovered and their validation is currently ongoing. The identification of genetic factors is of outmost clinical importance since there is a risk of transmission of genetic defects through natural or assisted reproductive techniques. The benefit of the genetic diagnosis of infertility has an obvious clinical significance for the patient itself with implications not only for his reproductive health but in many instances also for his general health.

Keywords Male infertility · Spermatogenesis · Genetics · Gene · Hypogonadism · Azoospermia · Oligozoospermia · Teratozoospermia · NGS · Exome

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List of Abbreviations

AIS	Androgen Insensitivity Syndrome
AOA	Artificial oocyte activation
ART	Assisted reproductive techniques
AZF	Azoospermia factor
CAIS	Complete Androgen Insensitivity
CAVD	Congentinal Absence of Vas Deferens
CBAVD	Congentinal Bilateral Absence of Vas Deferens
CFAP	Cilia and Flagella Associated Protein family
CHARGE	Coloboma, Heart defects, Atresia of choanae, Retardation of growth and/or development, Genital and/or urinary defects, Ear anomalies or deafness
CHH	Congenital hypogonadotropic hypogonadism
CPC	Chromosomal passenger complex
CPHD	Combined pituitary hormone deficiency
CUAVD	Congenital unilateral absence of vas deferens
DBD	DNA-binding domain
DFS	Dysplasia of fibrous sheath
DNA-DSB	DNA-Double Strand Breaks
DSD	Disorders of sexual development
D-WS	Dandy-Walker syndrome
FA	Fanconi anemia
FISH	Fluorescence in situ hybridization
GHS	Gordon Holmes syndrome
HS	Hartsfield syndrome
HTCA	Head-tail coupling apparatus
ICSI	Intracytoplasmic sperm injection
KS	Kallmann syndrome
LBD	Ligand-binding domain
MAIS	Mild androgen insensitivity
MGS	Morning glory syndrome
MMAF	Multiple Morphological Abnormalities of the sperm Flagella
NAHR	Nonallelic homologous recombination
NGS	Next-generation sequencing
NOA	Nonobstructive azoospermia
NTD	N-terminal domain
OA	Obstructive azoospermia
PAIS	Partial androgen insensitivity
PAR	Pseudoautosomal region
PCD	Primary ciliary dyskinesia
PCR	Polymerase chain reaction

PEPNS	Polyendocrine deficiencies and Polyneuropathies
PGD	Preimplantation genetic diagnosis
POI	Premature ovarian insufficiency
SHFM	Split-hand/foot malformation
SOD	Septo-optic dysplasia
TEM	Transmission electron microscopy
TESE	Testicular sperm extraction
WES	Whole-exome sequencing
WS	Waardenburg syndrome

16.1 Introduction

Infertility affects about 14% of the couples in the general population and globally male factor is contributing to it for about 50% of cases. In about 95% of cases, male factor implies quantitative or qualitative alterations of sperm parameters while in about 5% of cases it is related to semen deposition in vagina (aspermia, erectile dysfunction, retrograde ejaculation, etc.). Male infertility is a multifactorial complex pathological condition with highly heterogeneous phenotypic representations. Recently, male reproductive dysfunction has been classified into four etiologic categories: (1) hypothalamic–pituitary axis dysfunction; (2) quantitative alterations of spermatogenesis; (3) qualitative alterations of spermatogenesis; and (4) ductal obstruction/dysfunction (Tournaye et al. 2016). Genetic factors play an important role in each of these categories, with the highest prevalence in the severest form of quantitative alterations, i.e., azoospermia. In fact, karyotype (numerical and structural chromosomal anomalies) and Y chromosome microdeletions are found in about 15% of men affected by severe male factor infertility. In sharp contrast with the incidence of chromosomal anomalies, known monogenic alterations are relatively rare and their screening is restricted to congenital hypogonadotropic hypogonadism (CHH), absence of vas deferens, mild androgen insensitivity, and monomorphic terato/asthenozoospermia. In about 40% of quantitative disturbances of spermatogenesis, the etiology remains unknown and we refer to them as idiopathic infertility. Genetic factors are likely to play an important role in idiopathic testicular impairment/failure. A growing number of genes have been reported in idiopathic azoospermia/oligozoospermia but at the moment only a few of them have been validated by more than one study (Fig. 16.1). In this chapter, we are going to provide a detailed description of those genetic factors that are already included in the diagnostic workup of infertile men. In addition, a brief overview is given on genes with potential clinical interest.

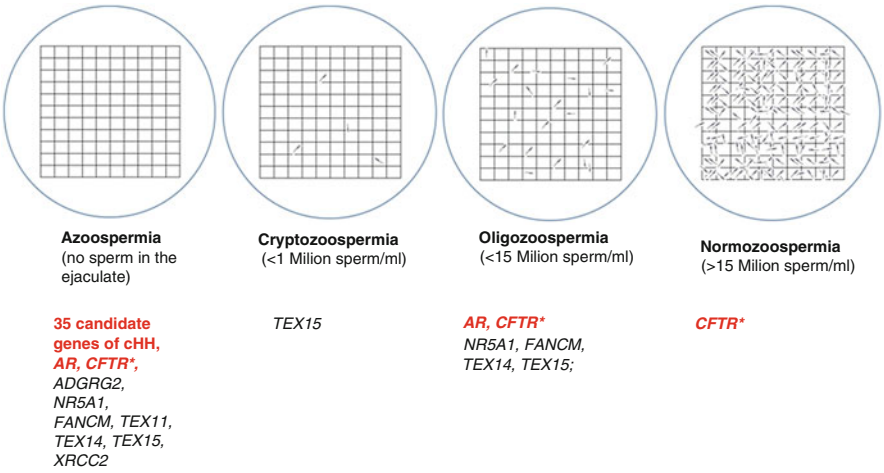

Fig. 16.1 Genotype–phenotype correlations, gene mutations versus semen phenotype. Genes in red with diagnostic value; genes in black with potential clinical value; *cHH* congenital Hypogonadotropic Hypogonadism, the complete list of genes is presented in Table 16.1. Asterisk mutations associated with unilateral congenital absence of vas deferens

16.2 Monogenic Causes with Diagnostic Value in the Four Etiologic Categories of Male Infertility

16.2.1 Genetic Causes of Hypothalamic–Pituitary Axis Dysfunction

A total of 35 candidate genes have been described in the literature to date (Boehm et al. 2015; Tournaye et al. 2016) with congenital hypogonadotropic hypogonadism (CHH) (Table 16.1). CHH is a rare, complex genetic disease (incidence of 1 in 8000 men) with variable expressivity, penetrance, and inheritance (Boehm et al. 2015). The classical phenotype of CHH is absent or delayed puberty, eunuchoid habitus, sparse or absent body hair, gynecomastia, cryptorchidism, micropenis, and very low testicular volume (<5 ml). However, in some cases, reduced spermatogenesis and mild hypoandrogenism are the only symptoms, resulting in a delayed CHH diagnosis after puberty. CHH can manifest itself with anosmia or hyposmia (Kallmann syndrome; KS) or as normosmic, isolated hypogonadotropic hypogonadism (Boehm et al. 2015). To note, KS can be associated with other developmental anomalies such as cleft lip or palate, dental agenesis, ear anomalies, congenital hearing impairment, renal agenesis, bimanual synkinesis, or skeletal anomalies (Boehm et al. 2015), whereas in normosmic CHH nonreproductive defects are absent. Some of the genes associated with CHH are also involved in different syndromic diseases (such as Gordon Holmes syndrome, CHARGE syndrome, and Waardenburg syndrome) (Boehm et al. 2015). Interestingly enough, "reversibility" of the gonadotropin deficiency after testosterone therapy has been described in about 10–15% of

Table 16.1 Monogenic causes with diagnostic value in the four major etiologic categories of male infertility

Etiology of reproductive impairment	Gene (OMIM)	Cytogenetic band	Other syndrome/ diseases	Protein function
Hypothalamic–pituitary axis dysfunction	*CHD7*[a] *(608892)*	8q12.2	CHARGE	Embryonic differentiation of GnRH neuron
	DUSP6[a] *(602748)*	12q21.33	–	
	FGF17[a] *(603725)*	8p21.3	D-WAS	
	FGF8[a] *(600483)*	10q24.32	CPHD	
	FGFR1[a] *(136350)*	8p11.23	CPHD, SOD, HS, SHFM	
	FLRT3 (604808)	20p12.1	–	
	HESX1 (601802)	3p14.3	CPHD, SOD	
	HS6ST1[a] *(604846)*	2q14.3	–	
	IL17RD (606807)	3p14.3	–	
	SOX10 (602229)	22q13.1	WS	
	SPRY4[a] *(607984)*	5q31.3		
	AXL (109135)	19q13.2	–	Migration of GnRH neurons
	CHD7[a] *(608892)*	8q12.2	CHARGE	
	FEZF1 (613301)	7q31.32		
	ANOS1 (300836)	Xp22.31		
	NSMF[a] *(608137)*	9q34.3		
	PROK2[a] *(607002)*	3p13		
	PROKR2[a] *(607123)*	20p12.3	CPHD; MGS	
	SEMA3A (603961)	7q21.11		
	SEMA3E (608166)	7q21.11		
	SEMA7A[a] *(607961)*	15q24.1		

(continued)

Table 16.1 (continued)

Etiology of reproductive impairment	Gene (OMIM)	Cytogenetic band	Other syndrome/ diseases	Protein function
	WDR11[a] *(606417)*	10q26.12	CPHD	
	TAC3 (162330)	12q13.3		Upstream and metabolic regulation of GnRH neuron function
	DMXL2 (612186)	15q21.2	PEPNS	
	KISS1 (603286)	1q32.1		
	KISS1R (604161)	19p13.3		
	LEP (164160)	7q32.1		
	LEPR (601007)	1p31.3		
	NR0B1 (300473)	Xp21.2		
	OTUD4 (611744)	4q31.21	GHS	
	PCSK1 (162150)	5q15		
	RNF216 (609948)	7p22.1	GHS	
	PNPLA6 (603197)	19p13.2	GHS	
	TAC3R (162332)	4q24		
	GnRH1 (152760)	8p21.2	–	GnRH synthesis
	GnRHR (138850)	4q13.2	–	GnRH receptor activation
Quantitative alterations of spermatogenesis	*AR (313700)*	Xq12	AIS	Steroid-hormone activated transcription factor
Qualitative alterations of spermatogenesis	*AURKC (603495)*	19q13.43		Chromosome alignment and segregation
	DNAH1 (603332)	3p21.1	PCD	Biogenesis of the axoneme
	DPY19L2 (613893)	12q14.2		Acrosome formation
	SUN5 (613942)	20q11.21		Anchoring sperm head to the tail

(continued)

Table 16.1 (continued)

Etiology of reproductive impairment	Gene (OMIM)	Cytogenetic band	Other syndrome/ diseases	Protein function
Ductal obstruction/ dysfunction	*CFTR (602421)*	7q31.2	Cystic fibrosis	Chloride transport

AIS Androgen Insensitivity Syndrome, *CHARGE* coloboma, heart defects, atresia of choanae, retardation of growth and/or development, genital and/or urinary defects, ear anomalies or deafness, *CPHD* combined pituitary hormone deficiency, *CTO* contributes to oligogenicity, *D-WS* Dandy-Walker syndrome; *GHS* Gordon Holmes syndrome, *HS* Hartsfield syndrome, *MGS* Morning Glory syndrome, *PCD* Primary Ciliary Dyskinesia, *PEPNS* polyendocrine deficiencies and polyneuropathies, *SHFM* split-hand/foot malformation, *SOD* septo-optic dysplasia, *WS* Waardenburg syndrome
[a]Gene responsible for both normosmic CHH and Kallmann syndrome

patients affected by KS or normosmic CHH (Quinton et al. 1999; Ribeiro et al. 2007; Raivio et al. 2007; Dwyer et al. 2016).

The 35 CHH genes are implicated either in the development/migration of the GnRH neurons or in the neuroendocrine regulation of GnRH secretion or action (Boehm et al. 2015). CHH presents a number of peculiar features from a genetic point of view: (1) in some cases the same gene (i.e., *FGFR1*, *PROKR2*) may cause both KS and normosmic CHH, implying that from a genetic point of view a clear distinction between the two clinical entities cannot be established; (2) it does not follow the rules of Mendelian inheritance since in about 20% of cases there is a digenic/oligogenic inheritance, i.e., two heterozygous mutations in two or more candidate genes.

16.2.1.1 Testing and Genetic Counseling

The indication for testing is restricted to patients with confirmed CHH after the exclusion of all secondary forms (pituitary tumors, empty sella, etc.). Currently, genetic testing is based on Next-Generation Sequencing (NGS) gene panel, which is able to provide the diagnosis in about 40% of cases (Boehm et al. 2015; Tournaye et al. 2016). Novel genes associated with CHH are expected to be discovered by whole-exome sequencing (WES) analysis in the near future.

Since in about 80% of CHH patients spermatogenesis can be induced by the administration of gonadotropins (Dwyer et al. 2015), gene mutations can be transmitted either spontaneously or by assisted reproductive techniques. Overall, the complexity of this disease (variable expressivity, penetrance, and inheritance pattern) makes predicting the exact health consequences for the offspring difficult. For this reason, the Preimplantation Genetic Diagnosis (PGD) or prenatal diagnosis should be offered to couples mainly for syndromic cases and for those cases where the gene mutation shows a clear-cut cause–effect relationship. A periodic suspension of the hormonal replacement therapy is recommended to all CHH patients in order to assess a potential recovery of the hypothalamic–pituitary axis. To note, genetic

testing does not help in identifying patients with higher probability of "reversal," since this condition has been described in association with mutations in different CHH candidates (Dwyer et al. 2016).

16.2.2 Genetic Causes of Quantitative Alterations of Spermatogenesis

16.2.2.1 *AR* (Androgen Receptor Gene)

AR (OMIM: 313700) is the only gene that is included in the diagnostic testing of specific cases of male infertility. Mutations in *AR* gene are associated with Androgen Insensitivity Syndrome (AIS) characterized by resistance to circulating testosterone. AIS is the most frequent cause of Disorders of Sexual Development (DSD) and based on the residual degree of functional capacity of the mutated AR the clinical phenotype can be divided into three categories: (1) Complete Androgen Insensitivity (CAIS; Morris syndrome) leading to a female phenotype in 46,XY individuals; (2) Partial Androgen Insensitivity (PAIS; Reifenstein syndrome) characterized by undervirilized male phenotype with ambiguous genitalia; (3) Mild Androgen Insensitivity (MAIS) associated with impaired sperm production in the presence of normal male genitalia (Krausz and Chianese 2014). Using conventional and Cre/Lox conditional *Ar*-null male mice recreated human disorders (Yeh et al. 2002). They showed female external sex development and testis atrophy with spermatocyte-stage arrest, resembling AIS human pathology. Sertoli cell-selective KO of the AR causes spermatocytic arrest, indicating an important role for intratesticular testosterone in meiosis (De Gendt et al. 2004).

The AR gene is situated on the X chromosome (Xq11–12) and contains eight exons that encode a protein of 920 amino acid residues. The protein functions as a steroid hormone-activated transcription factor and contains three major functional domains: the N-terminal domain (NTD, transcriptional activation region encoded by exon 1), the DNA-binding domain (DBD, encoded by exons 2 and 3), and the ligand-binding domain (LBD, encoded by exon 4–8). More than 1000 *AR* mutations have been described so far (Gottlieb et al. 2012) and the large majority of them are missense mutations located in the AR-DBD or AR-LBD leading to impairment in DNA or AR binding, respectively.

The *AR* gene also contains two polymorphic sites in the N-terminal transactivation domain (exon1) of the receptor: a polyglutamine tract -(CAG)*n*- and a polyglycine tract (GGC)*n*, which were the subject of many publications related to male infertility (Davis-Dao et al. 2007). The most extensively studied polymorphism concerns the trinucleotide CAG. Based on in vitro studies, it has been hypothesized that carriers of longer CAG repeat have a higher risk for infertility and cryptorchidism due to impaired androgen effect (Gao et al. 1996; Davis-Dao et al. 2007). This hypothesis has been challenged by novel functional and observational studies reporting that both a longer CAG tract and a shorter CAG tract might

have a negative effect on the receptor function; hence, an optimal number of CAG repeats are necessary for the highest transcription (Nenonen et al. 2011; Davis-Dao et al. 2012). We can speculate that the optimum range may vary between the genomic and non-genomic actions, and also in different tissues, because the effect of polyQ repeat on transactivation is cell-specific, presumably due to distinct profiles of co-regulator proteins (Krausz 2012).

Testing and Genetic Counseling

Given the variable clinical phenotypes, indications are different for each type of AIS. CAIS is suspected in case of a 46,XY woman with primary amenorrhea, normal breast development and pubertal growth, reduced or absent sexual hair, and absent female internal genitalia. Clinical management is complex and involves a multidisciplinary approach including psychologists, endocrinologists, urologists, and gynecologists. Because of malignancy risk, gonads, usually located in the abdomen or inguinal canal, are commonly removed, requiring subsequent estrogen replacement to maintain feminization. In case of PAIS, the phenotype is highly dependent on the degree of residual AR function, ranging from male-appearing genitalia to severe undermasculinization resembling female genitalia (Mongan et al. 2015). Management of severe forms of PAIS, including gender assignment, is rather complex. The hormone profile of AIS is typically represented by high Androgen Sensitivity Index (ASI), calculated as the product of serum testosterone x serum luteinizing hormone, i.e., high LH with relatively high testosterone levels. In hypoandrogenized infertile men with high ASI, *AR* testing is indicted. However, a routine screening to all infertile men is not advised, since the frequency of *AR* mutations in unselected infertile men varies from 0–1.7% (Ferlin et al. 2006; Rajender et al. 2007). The frequency of *AR* mutations in PAIS is 41%, whereas no official estimate is given in the available mutation databases for the MAIS phenotype (Gottlieb et al. 2012). The role of CAG repeats in male infertility is probably more complex than it has been previously proposed; there are still important unanswered questions such as: what range of *AR* CAG repeat lengths predisposes to impaired sperm production and what risk of infertility is associated with each length (Davis-Dao et al. 2007). These open questions limit the clinical use of (CAG)n testing.

16.2.2.2 Y Chromosome Linked Male Infertility

The Y chromosome contains genes essential for testis development and function, such as the genes residing in the azoospermia factor (AZF) regions and the master gene for testis determination (SRY; OMIM:480000). Y chromosome microdeletions, removing the entire AZF regions (complete deletions), are one of the leading causes of spermatogenic failure and the screening for AZF deletions became part of the routine diagnostic workup of men with severe oligozoospermia/azoospermia (Krausz et al. 2014). A peculiar feature of the boundary of the AZF regions is the presence of

repeated homologous sequences that are predisposed to deletion or duplication through a mechanism called nonallelic homologous recombination (NAHR). These deletions remove more than one gene in block; hence, they will not be further discussed in this chapter (for review see Krausz and Casamonti 2017). The only monogenic Y-chromosome-linked cause of male infertility concerns the *SRY* gene. *SRY* encodes the critical testis-determining transcription factor that activates a number of downstream transcription factors involved in testes formation. The gene is located below the pseudoautosomal region (PAR) of the short arm of the Y chromosome, and the erroneous translocation can occur during meiosis when the two sex chromosomes recombine between their PAR regions (Wu et al. 2014). This translocation leads to the 46,XX male syndrome (also known as de la Chapelle syndrome). This syndrome has a frequency of 1 in 20,000 children according to Genetics Home Reference (https://ghr.nlm.nih.gov/). Men with 46,XX male syndrome have smaller stature and a higher incidence of maldescended testes and gynecomastia, and are azoospermic with no exceptions (Vorona et al. 2007).

Testing and Genetic Counseling

Testicular sperm extraction (TESE) is not advised in XX male patients owing to the lack of Y chromosome-linked azoospermia factor (AZF) genes, meaning focal sperm production in the testis is not possible (Skaletsky et al. 2003). These patients can have hypoandrogenism, so a careful endocrine assessment (including analysis of FSH, LH, and testosterone levels) and follow-up monitoring of testosterone level are advised.

16.2.3 Genetic Causes of Qualitative Alterations of Spermatogenesis

16.2.3.1 *DPY19L2* (Dpy-19 Like 2 Gene)

DPY19L2 (OMIM: 613893) is the only gene included in the routine genetic diagnostic workup of globozoospermia. Globozoospermia is very rare, affecting 0.1% of infertile men, and is characterized by the production of round-headed, acrosome-less spermatozoa that are unable to fertilize the oocyte, as no acrosome reaction can occur (Fig. 16.2a). In mouse models, globozoospermia has been observed as a consequence of >50 different gene mutations (Coutton et al. 2015), but in humans, only mutations in the *DPY19L2* gene have been validated to be associated with this disorder. In fact, mutations in *DPY19L2* have been found in 60–80% of globozoospermic patients. *DPY19L2* is located on chromosome 12 and encodes a protein required during spermatogenesis for sperm head elongation and acrosome formation. The most frequent mutation is the complete deletion of the gene, caused by a similar mechanism to that observed for AZF deletions. *DPY19L2* is located in a region flanked by

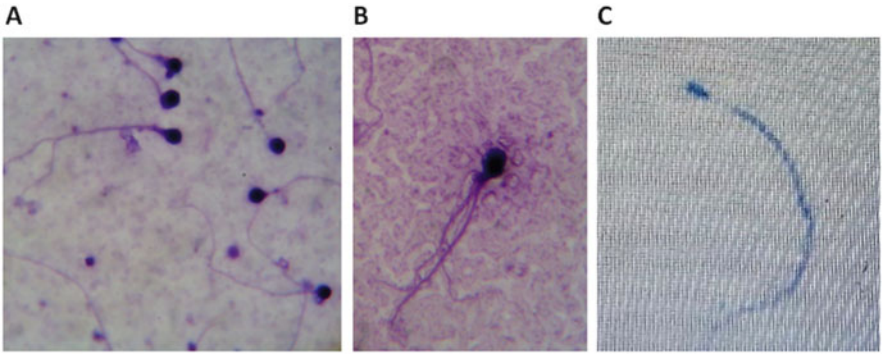

Fig. 16.2 Representative view of sperm morphology. (**a**) Roundheaded and acrosomeless spermatozoa. (**b**) Macrocephalic and multi-flagellated spermatozoa. (**c**) Acephalic spermatozoa

two 28 kb segmental duplications, which predisposes it to NAHR. The complete deletion of *DPY19L2* accounts for 80.4% of instances of *DPY19L2*-related globozoospermia, whereas the remaining instances are caused by intragenic deletions and point mutations (homozygous and compound heterozygous) (Ray et al. 2017).

Testing and Genetic Counseling

Mutations are mainly identified in patients with 100% globozoospermia; thus, genetic analysis should be restricted to this circumstance only. Given the high frequency of complete gene deletion, genetic testing can be easily performed using real-time quantitative PCR (Chianese et al. 2015) followed by breakpoint definition and mutation screening. Since *DPY19L2* deletions are not exceptionally rare in the general population (heterozygous carriers 1:85), screening in the female partners of male carriers prior intracytoplasmic sperm injection (ICSI) should be performed. The lack of phospholipase C-ζ, an acrosome phospholipase, is responsible for the absence of oocyte activation. Consequently, artificial oocyte activation (AOA) has been proposed as an option for patients with complete globozoospermia undergoing ICSI. However, the safety of AOA has been questioned as continued increases in intracellular calcium concentration can affect downstream molecular events, and it should be restricted to selected cases of 100% globozoospermia in which finding spermatozoa with residual acrosome is impossible (Kuentz et al. 2013).

16.2.3.2 *AURKC* (Aurora Kinase C)

To date, *AURKC* (OMIM: 603495) gene mutations are the only validated genetic causes macrozoospermia. Macrozoospermia, also known as sperm macrocephaly, affects <1% of the male population and it was reported for the first time in 1977 by Nistal and colleagues (1977). This qualitative disturbance is characterized by a high

percentage of spermatozoa with large, irregular heads and multiple flagella (Fig. 16.2b). *AURKC* gene is located in chromosome 19 and encodes for a component of the chromosomal passenger complex (CPC) in meiotic cells and is essential for correct meiotic chromosomal segregation and cytokinesis (Dieterich et al. 2007). The *AURKC* mutations are associated with alterations of meiotic divisions leading to tetraploid spermatozoa. The most common mutation is the deletion of a cytosine in the exon 3 (c.144delC), observed in more than 85% of patients affected by macrozoospermia (Ray et al. 2017). Interestingly enough, the mutation is relatively common in heterozygosis in the Maghrebian population (1/50 men) and it has been proposed that heterozygote carriers may have a selective advantage due to a more relaxed meiotic checkpoint (Ben Khelifa et al. 2012). In Europeans, a recurrent stop gain mutation in exon 6 (p.Y248∗) has been described (Ben Khelifa et al. 2012).

Testing and Genetic Counseling

All men with macrozoospermia should be tested for *AURKC* mutations before undergoing Assisted Reproductive Techniques (ART). After genetic testing, two different scenarios can occur: identification of homozygous or compound heterozygous mutations or an absence of mutations in this gene. In the first scenario, ICSI is not advised even after motile sperm organelle morphology examination, as all spermatozoa are polyploid (and are mostly tetraploid) and, therefore, normal embryonic development is not possible. By contrast, ART is not contraindicated in patients without mutations, but sperm FISH should be performed to evaluate the proportion of euploid sperm; hence, the likelihood of success. PGD can be proposed to those with intermediate rate of aneuploid spermatozoa.

16.2.3.3 *DNAH1* (Dynein Axonemal Heavy Chain 1)

DNAH1 gene (OMIM: 603332) mutations seems to be the major cause of Multiple Morphological Abnormalities of the sperm flagella (MMAF) (Ben Khelifa et al. 2014; Amiri-Yekta et al. 2016; Wang et al. 2017; Sha et al. 2017a; Tang et al. 2017; Coutton et al. 2018). MMAF, previously reported as dysplasia of fibrous sheath (DFS), is a rare disease defined as an asthenoteratozoospermia resulting from a mosaic of morphological abnormalities concerning the sperm flagella, including absent, coiled, bent, angulated, irregular, or short flagella (Ben Khelifa et al. 2014). In addition, lack of central microtubules and/or dynein arms may also be observed by transmission electron microscopy (TEM) in the sperm flagella of the affected subjects (Ben Khelifa et al. 2014). The incidence of MMAF has not already been investigated precisely. *DNAH1* is located on chromosome 3 and encodes an axonemal inner dynein arm heavy chain and when it is absent, the axoneme is grossly disorganized, often lacking the central pair (9 + 0 structure). Biallelic *DNAH1* mutations seem to be responsible for 30% of MMAF patients (Coutton et al. 2018).

Testing and Genetic Counseling

The screening for *DNAH1* mutations is recommended in patients affected by severe to complete asthenozoospermia due to sperm flagellar alterations. Flagellar abnormalities have been reported to be associated with an elevated frequency of aneuploidies and a poor ICSI outcome (Lewis-Jones et al. 2003; Baccetti et al. 2005; Collodel and Moretti 2006; Ghedir et al. 2014). However, patients with MMAF with mutated *DNHA1* showed low aneuploidy rate and normal sperm DNA integrity, indicating that not all patients with MMAF are at risk of chromosomal anomalies (Wambergue et al. 2016).

In 2015, a homozygous mutation in *DNAH1* was observed in two sisters affected by Primary ciliary dyskinesia (PCD) (Imtiaz et al. 2015), which is a disorder characterized by chronic respiratory tract infections, abnormally positioned internal organs, and infertility. This observation has prompted the novel hypothesis of a 'phenotypic continuum' ranging from infertile patients with PCD to patients with MMAF with no or mild PCD manifestations (Ray et al. 2017). Given the multitude of genes involved in ciliagenesis and function, MMAF could be a phenotypic variant of the classical form of PCD, and mutations affecting sperm flagella could be compensated for by other genes involved in other ciliated tissues. Therefore, it is still unclear the exact health consequences for the offspring and whether the female partner should be screened for *DNAH1* mutations.

16.2.3.4 *SUN5* (*Sad1* and *UNC84* Domain Containing 5 Gene)

The phenotype of the *SUN5*-mutated patients is characterized by acephalic spermatozoa with a variable but low proportion of abnormal head–tail junctions and tailless heads (Shang et al. 2017) (Fig. 16.2c). This sperm defect is due to the failure of centriole-tail attachment to the spermatid nucleus during the last phase of spermatogenesis. *SUN5* (OMIM: 613942) encodes a testis-specific protein localized in the neck region of spermatids. The disease is extremely rare and it is transmitted through recessive inheritance. Homozygous and compound heterozygous mutations were reported by four different authors (Zhu et al. 2016; Elkhatib et al. 2017; Shang et al. 2017; Sha et al. 2018b).

Testing and Genetic Counseling

Patients affected by acephalic spermatozoa should be screened for *SUN5* mutations. The only option for a biological paternity is ICSI through the selection of tailless sperm heads. The majority of papers report no pregnancy despite the presence of fertilized eggs. In five articles, nine couples obtained pregnancy after repeated ICSI attempts (Kamal et al. 1999; Porcu et al. 2003; Emery et al. 2004; Gambera et al. 2010; Shang et al. 2017).

16.2.4 Genetic Causes of Ductal Obstruction

16.2.4.1 *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator Gene)

Mutations in *CFTR* (OMIM: 602421) have been largely described in patients affected by Congenital Absence of Vas Deferens (CAVD). The CAVD may occur either as an isolated reproductive disorder or as an atypical symptom of Cystic Fibrosis, and accounts for up to 25% of patients with Obstructive Azoospermia (OA) (Oates and Amos 1994). It may affect one (CUAVD) or both vas deferens (CBAVD). The CUAVD is a rare condition associated with either oligo/or normozoospermia. In contrast, CBAVD associated with agenesis of seminal vesicles is characterized by typical semen alterations, such as low semen volume (<1 ml) with an acid pH (<7) and absence of spermatozoa. The *CFTR* gene is located on chromosome 7q31.2, contains 27 exons (Kerem et al. 1989; Riordan et al. 1989), and encodes a protein involved in chloride conduction across epithelial cell membranes. To date, more than 2000 variants have been identified in *CFTR* gene (http://www.genet.sickkids.on.ca/Home.html) and they are categorized in severe and mild mutations depending on their functional consequences. Although geographical and ethnic differences have been demonstrated in *CFTR* mutations, the most common mutations in CBAVD patients are F508del, 5T, and R117H (Yu et al. 2012). 5T is the shortest allele of IVS8-(T)*n*, which is a length variant of a polypyrimidine tract at the splice acceptor site of intron 8 of the *CFTR* gene. The length of the T tract (IVS8-5T, IVS8-7T, IVS8-9T) affects the splicing efficiency of exon 9 and thus the amount of normal *CFTR* mRNA. The phenotypic penetrance of 5 T allele depends on the length of adjacent TG repeats (12 or 13) and the M470 V missense mutation in exon 10, i.e., the 12TG-5T-V470 haplotype increases the risk of having CBAVD (de Meeus et al. 1998; Du et al. 2014).

Testing and Genetic Counseling

The screening for CFTR gene mutation is recommended in subjects with CAVD without renal agenesis (Jungwirth et al. 2012). In fact, subjects with CAVD and renal agenesis (in the majority of cases of unilateral agenesis of vas deferens) are considered to have different genetic basis, which may be attributed to defect of mesonephric duct development in the embryo (McCallum et al. 2001). This fact implies that all patients affected by CAVD should undergo an ultrasound scan of the pelvic region prior to genetic testing. Routine screening for *CTFR* variants is based on a panel of mutations (30–50 mutations) that are the most common for a given ethnic population (de Souza et al. 2017). In instances in which the two mutations are not identified using this panel (as CAVD is a recessive disease), the whole *CFTR* gene is subjected to sequencing in order to search for the second mutation. In those patients in whom pathogenic variants have been identified, genetic counseling is mandatory since

patients with CBAVD are assumed to have normal testicular function, and they can undergo TESE–ICSI (as azoospermia is caused by obstruction) and generate their own biological children. Given that the carrier frequency of *CFTR* mutations in people of European descent is high (1 in 25), screening of the partner is mandatory in order to evaluate the risk of giving birth to a child affected by cystic fibrosis. If both parents are carriers, prenatal or PGD should be undertaken.

16.3 Additional Monogenic Causes of Male Infertility with Potential Clinical Interest

The diffusion of NGS platforms is allowing the identification of a number of genes involved in various semen phenotypes. However, only few of them have been validated by more than one independent study on a relatively high number of subjects (Table 16.2). In the following paragraphs we briefly describe those genes that were validated by more than one study and are potential candidates for diagnostic testing in the future.

16.3.1 Validated Candidate Genes Involved in Quantitative Alterations of Spermatogenesis

A total of six genes leading quantitative impairment of spermatogenesis have been validated by more than one independent study: *NR5A1* (OMIM: 184757), *TEX11* (OMIM: 300311), *TEX14* (OMIM: 605792), *TEX15* (OMIM: 605795), *FANCM* (OMIM: 609644), and *XRCC2* (OMIM: 600375). With the exception of *TEX11*, which is X-linked, the remaining genes are mapping to autosomes. Apart from *NR5A1*, which follows the Autosomal Dominant inheritance pattern, only recessive mutations lead to quantitative alterations of spermatogenesis.

NR5A1 encodes steroidogenic factor 1 (SF1), crucial in male and female gonadal development and steroidogenesis. Heterozygosis mutations in *NR5A1* have been associated with a variety of phenotypes ranging from primary adrenal insufficiency (AI) and complete 46,XY gonadal dysgenesis (Schimmer and White 2010; Ferraz-de-Souza et al. 2011) to 46,XY DSD including bilateral anorchia (Philibert et al. 2007; Brauner et al. 2011), hypospadias (Allali et al. 2011; Brandt et al. 2013), and hypogonadotropic hypogonadism (Hu et al. 2012).

Heterozygous mutations have also been reported in patients affected by severe oligozoospermia or azoospermia (Bashamboo et al. 2010; Ropke et al. 2013; Zare-Abdollahi et al. 2015; Ferlin et al. 2015; Tuttelmann et al. 2018) with a frequency ranging from 0.6% (Ropke et al. 2013) to 2.5% (Tuttelmann et al. 2018).

Concerning *TEX11*, *TEX14*, and *TEX15*, they belong to the family of Testis Expressed genes and, as their name indicates, they are over- or specifically expressed

Table 16.2 Monogenic causes of male infertility with potential clinical interest, validated in more than one study population

Etiology of reproductive impairment	Gene (OMIM)	Cytogenetic band	Cohort type	Associated semen/testis phenotype(s)	Genotype in affected individuals	References
Quantitative alterations of spermatogenesis	*FANCM (609644)*	14q21.2	Consanguineous family	NOA + Oligoasthenozoospermia	Homozygosis	Yin et al. (2019)
			Non-consanguineous family + sporadic cases	NOA	Homozygosis/ compound heterozygosis	Kasak et al. (2018)
	NR5A1 (184757)	9q33.3	Sporadic cases	NOA + Oligozoospermia	Heterozygosis	Bashamboo et al. (2010)
			Sporadic cases	NOA + Oligozoospermia	Heterozygosis	Ropke et al. (2013)
			Sporadic cases	NOA	Heterozygosis	Zare-Abdollahi et al. (2015)
			Sporadic cases	NOA + Oligozoospermia	Heterozygosis	Ferlin et al. (2015)
			Sporadic cases	NOA	Heterozygosis	Tuttelmann et al. (2018)
	TEX11 (300311)	Xp11	Sporadic cases	NOA	Hemizygosis	Yatsenko et al. (2015)
			Non-consanguineous family + sporadic cases	NOA	Hemizygosis	Yang et al. (2015)
			Non-consanguineous family	NOA	Hemizygosis	Sha et al. (2018a)
			Sporadic cases	NOA	Hemizygosis	Tuttelmann et al. (2018)
	TEX14 (605792)	17q22	Consanguineous family	NOA	Homozygosis	Gershoni et al. (2017)
			Consanguineous family + sporadic cases	NOA	Homozygosis	Fakhro et al. (2018)

	TEX15 (605795)	8p12	Consanguineous family	NOA+ Severe oligozoospermia	Homozygosis	Okutman et al. (2015)
			Non-consanguineous family	NOA	Compound heterozygosis	Colombo et al. (2017)
			Consanguineous family	Cryptozoospermia	Homozygosis	Wang et al. (2018)
	XRCC2 (600375)	7q36.1	Consanguineous family + sporadic cases	NOA	Homozygosis	Yang et al. (2018)
			Consanguineous family	NOA	Homozygosis	Zhang et al. (2019)
Qualitative alterations of spermatogenesis	*CFAP43 (617558)*	10q25.1	Consanguienous families + sporadic cases	MMAF	Compound heterozygosis	Tang et al. (2017)
			Consanguineous family + non consanguineous family + sporadic cases	MMAF	Homozygosis	Sha et al. (2017b)
			No data[a]	MMAF	Homozygosis/ compound heterozygosis	Coutton et al. (2018)
	CFAP44 (617559)	3q132	Consanguienous families + sporadic cases	MMAF	Homozygosis	Tang et al. (2017)
			Consanguineous family + non-consamnguineous family + sporadic cases	MMAF	Homozygosis and compound heterozygosis	Sha et al. (2017b)
			No data[a]	MMAF	Homozygosis	Coutton et al. (2018)
	CFAP69 (617949)	7q21.13	No data[a]	MMAF	Homozygosis/ compound heterozygosis	Dong et al. (2018)
			Consanguineous family + sporadic cases	MMAF	Homozygosis	He et al. (2019)

(continued)

Table 16.2 (continued)

Etiology of reproductive impairment	Gene (OMIM)	Cytogenetic band	Cohort type	Associated semen/testis phenotype(s)	Genotype in affected individuals	References
	WDR66 (618146)	12q21.31	No data[a]	MMAF	Homozygosis	kherraf et al. (2018)
			Consanguienous families + sporadic cases	MMAF	Homozygosis and compound heterozygosis	Li et al. (2019)
			Consanguineous family + sporadic cases	MMAF	Homozygosis and compound heterozygosis	Auguste et al. (2018)
	PMFBP1 (618085)	16q22.2	Consanguineous families + sporadic cases	Acephalic spermatozoa	Homozygosis/ compound heterozygosis	Zhu et al. (2018)
			Consanguineous family + sporadic case	Acephalic spermatozoa	Homozygosis/ compound heterozygosis	Sha et al. (2019)
	SPATA16 (609856)	3q23.31	Consanguineous family	Globozoospermia	Homozygosis	Dam et al. (2007)
			Sporadic cases	Globozoospermia	Homozygosis	ElInati et al. (2016)
Ductal obstruction/ dysfunction	*ADGRG2 (300572)*	Xp22.13	Sporadic cases	CBAVD	Hemizygosis	Patat et al. (2016)
			Sporadic cases	CBAVD	Hemizygosis	Yang et al. (2017)
			Non-consanguienous family	CBAVD	Hemizygosis	Khan et al. (2018)

NOA nonobstructive azoospermia, *MMAF* Multiple Morphological Abnormalities of the sperm Flagella, *CBAVD* congenital bilateral absence of vas deferens
[a]Not precise data about but the type of cohort analyzed; however, they note that the majority of patients come from consanguineous families

in the testis. While *TEX15* is not associated with a clear-cut semen phenotype, since it has been found mutated both in patients with NOA and crypto/oligozoospermia (Okutman et al. 2015; Colombo et al. 2017; Wang et al. 2018), mutations in *TEX11* and *TEX14* are restricted to NOA (Yatsenko et al. 2015; Yang et al. 2015; Gershoni et al. 2017; Tuttelmann et al. 2018; Sha et al. 2018a; Fakhro et al. 2018). More precisely, *TEX11* mutations seem to be more frequent in NOA men with testis histology of meiotic arrest (Yatsenko et al. 2015) and should be tested in cases of suspected meiotic arrest.

FANCA (OMIM:607139), *FANCM*, and *XRCC2* are part of the Fanconi Anemia (FA) gene family. Proteins encoded by the FA gene family are involved in (DNA double strand breaks) DSB repair and are essential for mitosis and meiosis. By performing exome analysis, recessive *FANCA* mutations have been described in men affected by NOA (Sertoli Cell Only syndrome) and mild/borderline hematological alterations (Krausz et al. 2019). This finding underlies the importance of considering not only hormone dosage but also hematological parameters in the diagnostic workup of SCOS patients. Diagnosing occult FA is highly relevant since it is a medical condition that predisposes to specific FA-related cancers.

Mutations in *FANCM* are not associated with bone marrow failure and have been identified in both NOA and oligoasthenozoospermia (Kasak et al. 2018; Yin et al. 2019). Meiosis-specific mutations in the *XRCC2* gene were reported in NOA patient with spermatocytic arrest (Yang et al. 2018; Zhang et al. 2019). Interestingly enough, mutations in this gene also cause Premature Ovarian Insufficiency (POI) in females (Zhang et al. 2019). Hence, the genetic counseling should involve not only male but also female relatives in case of *XRCC2* mutations.

16.3.2 Validated Candidate Genes Involved in Qualitative Alterations of Spermatogenesis

Monomorhpic teratozoospermia defines a group of rare morphological anomalies, mainly globozoospermia, MMAF and sperm acephalia. The large majority of cases occur in consanguineous families since these are recessive diseases. In globozoospermia, besides the *DPY19L2* gene (see paragragh on routine screening), mutations in *SPATA16* (OMIM: 609856) have been reported in two independent studies (Elinati et al. 2016; Dam et al. 2007). This gene is located on chromosome 3 and encodes a testis-specific protein belonging to the tetratricopeptide repeat-like superfamily. The encoded protein localizes to the Golgi apparatus and is involved in the formation of sperm acrosome. Concerning the MMAF phenotype, besides *DNAH1* mutations, homozygous, and compound heterozygous mutations in *CFAP43* (OMIM: 617558), *CFAP44* (OMIM: 617559), *CFAP69* (OMIM: 617949), and *WDR66* (OMIM: 618146) have been reported in more than one study (Sha et al. 2017b; Tang et al. 2017; Coutton et al. 2018; Dong et al. 2018; Auguste et al. 2018; Kherraf et al. 2018; He et al. 2019). These genes encode a

family of proteins belonging to the cilia and flagella associated protein family (CFAP) and are necessary to produce functional flagella. Interestingly enough, *CFAP43* and *CFAP44*, *WDR66* encode WD-repeat proteins, which confirm the importance of these type of proteins in human diseases and especially in male infertility. Overall, mutations in these genes may explain up to 50% of cases of MMAF (Li et al. 2019). Finally, in relationship with acephalic spermatozoa two independent studies reported homozygous and compound heterozygous mutations in *PMFBP1* (OMIM: 618085) (Zhu et al. 2018; Sha et al. 2019). PMFBP1 is localized at the head–tail coupling apparatus (HTCA) and cooperates with SUN5 and SPATA6 to connect sperm head to tail (Zhu et al. 2018). It has been postulated that mutations in *SUN5* and *PMFBP1* may explain 70% of cases of acephalic spermatozoa syndrome (Zhu et al. 2018); however, since mutations in *PMFBP1* have been only found in patients from Asia, further validation in other ethnic populations is needed.

16.3.3 Validated Candidate Genes Involved in Ductal Obstruction

After a comprehensive analysis of the *CFTR* gene, in about 20% of cases the origin of CAVD remains unknown. Recently, the *ADGRG2* gene (OMIM: 300572) has been identified as a new candidate gene in CBAVD in three independent studies (Patat et al. 2016; Yang et al. 2017; Khan et al. 2018). *ADGRG2* is an X-linked gene encoding an adhesion-class G protein-coupled receptor and is highly expressed in the efferent ducts (Obermann et al. 2003). Adgrg2-mutant mice develop fluid accumulation in the testes ducts, leading to an obstructive infertility phenotype, which resembles that described in men with *ADGRG2* mutation (Davies et al. 2004). Pathogenic *ADGRG2* variants were reported, accounting for 11%–15% of the CBAVD patients who are *CFTR*-negative (Patat et al. 2016; Yang et al. 2017).

16.4 Conclusions

Although more than 2000 genes have been predicted to be involved in human spermatogenesis, there are relatively few monogenic mutations that have been conclusively demonstrated and validated to cause male infertility in humans. Consequently, the current diagnostic genetic testing is restricted to a relatively small set of genes. Genetic counseling of the couple is an absolute requirement prior to assisted reproduction and in some instances includes testing of the female partner and recommendation for PGD. The major breakthrough in the discovery of genetic factors involved in male infertility occurred more than 30 years ago with the identification of the Y-chromosome-linked AZF region deletions (Vogt et al.

1996), which implied the deletion in block of more than one gene. While candidate gene re-sequencing studies were relatively successful in uncovering the genetic basis of CHH, this approach did not lead to novel diagnostic tests in idiopathic oligo/azoospermia (Krausz and Riera-Escamilla 2018). Next-generation sequencing allowing the simultaneous analysis of several thousands of genes or the entire exome has contributed to major advances in the genetic diagnosis of monomorphic teratozoospermia, cHH, and in familial cases of idiopathic azoospermia. Exome studies are currently ongoing in quantitative impairment of spermatogenesis in large cohorts of sporadic patients. These studies have the potential to provide novel genetic diagnostic tests also in this category of patients. The clinical impact of discovering such factors became even more important in the era of in vitro fertilization, since these patients can now generate their own biological child through these techniques and the identification of transmissible genetic causes has relevance for their future children. Besides the consequences on reproductive health, an emerging issue is the possible genetic link between idiopathic impaired spermatogenesis and higher morbidity and mortality rates. This important topic is currently addressed by androgeneticists and will allow a more holistic clinical evaluation of our infertile patients.

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