Research Article

Male Infertility and Genetic screening: Guidelines in 2021

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Abstract

For many years, genetic screening for male infertility was limited to a few analyses: karyotyping, screening for Y microdeletions, and tests for the most frequent cystic fibrosis transmembrane conductance regulator (*CFTR*) gene variants. The development of new technologies, such as chromosome microarray or new genome sequencing, has broadened access to whole-genome analyses. Over the last decade, many genetic defects have been described, and new strategies seem to emerge. Hence, by focusing on peripheral (rather than central) failures of spermatogenesis, the objectives of the present study were to review the latest data on clinical practice (rather than the physiopathology of these genetic abnormalities) and suggest new guidelines for the genetic screening of male infertility.

Introduction

The World Health Organization (WHO) considers infertility (defined as the inability to conceive after 12 months of sexual intercourse without the use of contraceptives) to be a major health concern. In about half of these couples, infertility is of male origin [1]. Semen analysis is the first-line test for infertile couples and can often reveal congenital or acquired

causes of male infertility; these notably include quantitative and/or qualitative abnormalities in spermatogenesis, which affect the sperm count, motility and/or morphology. These abnormalities lead to oligo/azoospermia, asthenospermia, and teratozoospermia, respectively. 47,XXY aneuploidy diagnosis for the Klinefelter syndrome [2], karyotype analysis has been proposed to infertile patients, and many series have reported other types of chromosome rearrangements [3]. Many of these anomalies involve the sex chromosomes or autosomal Robertsonian translocations. Lower the sperm count, higher the frequency of chromosomal anomalies; this is mainly due to Klinefelter syndrome, which is observed in 15% of men with azoospermia. Karyotyping revealed that Y chromosome rearrangements - especially those involving long arm deletions - are associated with infertility. It was suggested that these conditions were due to an azoospermic factor (AZF), and three regions (AZFa, AZFb and AZFc) were subsequently identified [4]. During the same period, it was found that almost all men with cystic fibrosis (CF) had a congenital bilateral absence of the vas deferens (CBAVD). It has further been hypothesized that isolated CBAVD (OMIM#277180) is due to a distinct genetic entity associated with an elevated frequency of CF gene mutations [5] – now known as *CFTR*-related disease. Until that period, many studies had reported an association between genetic polymorphisms and male infertility but routine clinical applications of this knowledge were lacking. In parallel, a variety of different syndromes and single nucleotide variants (SNVs) associated with male infertility were described but the vast majority of these variants were private. With the emergence of whole-genome molecular analyses and the assessment of cohorts of men with homogeneous teratozoospermia, a number of autosomal recessive

causes have been reported. Firstly, a homozygous SNV in the gene coding for aurora kinase C (*AURKC*) was reportedly responsible for most cases of macrozoospermia in a population of consanguineous men from North Africa [6]. Later, some men with globozoospermia were found to have a homozygous deletion of the dpy-19-like 2 gene (*DPY19L2*), or compound heterozygotes for *DPY19L2* defects [7]. Initially, the strategy was based on a candidate gene approach that combined SNP array analysis with conventional molecular biology. The paradigm for genetic testing has now been changed totally by the development of next-generation sequencing technologies, such as whole-exome sequencing. At present, many SNVs have been reported in men with multiple morphological abnormalities of the flagella (MMAF), azoospermia, and other disorders. However, in contrast to macrozoospermia and globozoospermia (where *AURKC* and *DPY192* mutations in sperm account for most of the genetic defects), other syndromes are genetically heterogeneous. Hence, it appears to be necessary to define genetic testing guidelines as a function of the sperm phenotype, with a view to determining the etiology of these male infertilities and then choosing the best treatment strategy.

Quantitative defects

Azoospermia

Azoospermia is defined as the total absence of spermatozoa in the ejaculate in two successive semen examinations. It accounts for around 10% of cases of male infertility, and affects about 1% of the men in the general population [8-10]. The condition can be classified as non-obstructive azoospermia (NOA, associated with spermatogenesis failure, and accounting for 60% of cases) or obstructive azoospermia (OA, characterized by normal

spermatogenesis and an obstruction in the seminal tract, and accounting for the remaining 40%) [11,12].

In almost all cases of azoospermia, the combination of testicular sperm extraction (TESE) with *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) gives the patient an opportunity to become a father [13].

Sperm can be retrieved successfully from more than 95% of *men with OA*. The most challenging question for men with congenital bilateral or unilateral absence of the vas deferens (OMIM #277180) is the need to fully sequence the *CFTR* gene in both the man and his partner, in order to evaluate the risk of CF in the offspring [14]. Given that almost 80% of men with CBAVD carry one or two *CFTR* mutations [14], it is impossible to consider aTESE before genetic testing and counseling. Furthermore, a general check-up on the patient's respiratory and pancreatic functions appears to be useful. However, a quarter of men lacking a known *CFTR* mutation have a defect in the *AGDRG2* gene associated with X-linked CBAVD (OMIM #300985) [15]. No recurrent *CFTR* and *ADGRG2* mutations have been reported, however, although various candidate genes (such as *PANK2* and *SLC9A3*) have been suggested in the literature [16].

For *men with NOA*, the sperm retrieval rate is around 40 to 50%. Many genetic defects are associated with this condition, and sperm extraction may be contraindicated by the results of genetic testing in some of these. The first-line analysis is based on karyotyping and Y chromosome microdeletion screening [3,17]. A 46,XX karyotype (usually 46,X,der(X)t(X;Y)(p22.3;p11.2) results from an unbalanced *de novo* X-Y translocation and the translocation of the sex-determining region of the Y

chromosome to the X chromosome) contraindicates TESE. The second one contraindication is AZFa and/or AZFb microdeletion, leading respectively to Sertoli-cell-only syndrome and sperm maturation arrest [18]. Although other chromosome abnormalities (such as Klinefelter syndrome), do not constitute a contraindication to TESE, genetic counselling is required to evaluate the risk of an unbalanced karyotype in the offspring; this mainly applied to reciprocal or Robertsonian translocations and inversions. Karyotyping leads to a diagnosis in more than 15% of cases, and so it has been hypothesized that most of the genetic causes of male infertility have yet to be characterized - probably because of the large number of genes involved [19]. The emergence of whole-exome sequencing has led to a great increase in the number of different gene defects reported [16]. Initially, only *TEX11* (an X linked coding gene) was reported recurrently reported in the literature. However, recurrent abnormalities in genes such as *SYCE1*, *MEI1*, *STAG3*, *TEX14*, and *TEX15* have now been described [20,21]. Many of the latter (except TEX11) have been identified in consanguineous families, and are meiosis-specific and are observed in men with sperm maturation arrest, a condition in which sperm cannot be retrieved by TESE. In view of (i) the development of genetic analysis software that facilitates the interpretation of test results and (ii) decreases in the cost of whole-exome sequencing, whole-exome sequencing analysis needs to be rapidly implemented in clinical practice - especially for consanguineous men, after karyotyping and Y chromosome microdeletion screening. The identification of gene defects also facilitates a discussion of the risk/benefit balance of TESE with the patient.

Oligospermia

For men with a low sperm count, karyotyping alone should be suggested as a guide to the etiology. Subsequent genetic counseling can evaluate the risk for the offspring (see the previous paragraph) as a function of the type of chromosomal segregation during meiosis.

Quantitative defects

Teratozoospermia

Here, we only considered homogeneous teratozoospermia, i.e. conditions in which more than 99% of spermatozoa are affected: macrozoospermia, globozoospermia, acephalic sperm, and MMAF.

Macrozoospermia

Macrozoospermia (MIM # 243060, also referred to as macrocephalic sperm head syndrome) was first described in 1977 [22]. The spermatozoa have large, abnormally shaped heads, and multiple flagella (usually four). The condition leads to primary infertility, with no chance of paternity. All spermatozoa are aneuploid, with 96 chromosomes in general – regardless of the technique used for sperm selection [23]. According to the literature data, this syndrome is due to *AURKC* mutations. In North African populations, a founding event led to a recurrent missense SNV (c.144delC) [6]. A few other mutations have been detected elsewhere in the world, with a recurrent c. p.Y248* SNV in the European population, for example [24]. At present, genetic screening for *AURKC* mutations is recommended for men in whom all sperm are macrocephalic, with a focus on particular recurrent mutations as a function of the ethnic origin. Whole-exome sequencing is only recommended for syndromic men in whom 2 deleterious SNVs have not been identified. For men with homozygous or compound heterozygous mutations in *AURKC* (deleterious SNVs), only sperm

donation or adoption is possible. Hence, the genetic diagnosis may help the patient to weigh up the various options. Genetic screening is not useful for men with a diagnosis of inhomogeneous teratozoospermia. For men with some normally shaped sperm and in whom no mutation has been found, the results of a sperm FISH analysis and a sperm DNA decondensation assay will give an idea of the chances of paternity [25].

Globozoospermia

Globozoospermia (MIM 613958, a severe teratozoospermia with primary infertility) was first described in humans in 1971 [26]. It is characterized by round spermatozoa that lack an acrosome. Hence, the spermatozoa are unable to adhere to and penetrate the zona pellucida. There is a chance of fatherhood with ICSI, although the fertilization rate in globozoospermia is low because of the absence of phospholipase C zeta (PLCz) and thus a lack of oocyte activation after injection [27]. Acrosome biogenesis is mechanistically complex, and only a few related gene defects have been identified. More than 90% of the patients described in the literature have a *DPY19L2* defect – a homogenous deletion in 80% of cases. Men with globozoospermia should first be screened for the homozygous *DPY19L2* deletions. If none are found, whole-exome sequencing (including *DPY19L2* and all the other genes previously described) appears to be justified. In a recent study, this strategy enabled a diagnosis in 75% of men in whom more than 50% of the sperm in the ejaculate were abnormal [28].

Acephalic sperm

Acephalic spermatozoa syndrome (MIM 617187) is a rare condition that was first described in 1979 [29]. The sperm are predominantly headless or lack flagella [30]. In contrast to the syndromes described

above, a large variety of genetic abnormalities have been described - although defects in the *SUN5* gene appears to be frequent [31]. Given that defects in many genes have been reported, it seems sensible to use first-line whole-genome sequencing. Some gene defects are reportedly associated with a lower chance of fatherhood. Hence, if more data are necessary, genetic screening should be performed before the ICSI procedure.

Multiple morphological abnormalities of the flagella

Although MMAF is a rare syndrome, cases have been reported regularly since 1984 [32]. Due to peri-axonemal and axonemal defects, the flagella of the sperm in the ejaculate are short, coiled, absent or of irregular caliber. It has recently been demonstrated that MMAF is genetically heterogeneous and can result from defects in more than 20 genes [33]. Hence, whole-exome sequencing appears to be necessary in these men. The diagnosis is purely etiologic and does not have any impact on clinical practice. The take-home baby rate after ICSI for MMAF is similar to that observed in the whole patient population.

Other situations

Male infertility is not limited to defects in the sperm count, motility or morphology defect. As with mutations in the gene coding for PLCz [34], infertility is sometimes due to fertilization failure. In this rare, idiopathic situation (i.e. in the apparent absence of sperm or female defects), a whole-exome analysis might be a good opportunity for explain the inability to conceive.

In conclusion, the discovery of a sperm defect should prompt the initiation of genetic screening based on

whole-exome sequencing - now a diagnostic tool that is easily accessible, as a first or second-line genetic test (Table 1). However, whole-exome sequencing should always be preceded by genetic counselling. The purpose of the analysis must be clearly explained to the patient because gene defects unrelated to infertility (e.g. predispositions to cancer or early-onset neurodegenerative diseases) could potentially be identified.

	First-line genetic test	Second-line genetic test	**Changes in clinical practices after genetic testing**
NOA	Karyotyping and Y chromosome microdeletion	Whole-genome analysis	1: Chromosome rearrangement: considered PGD or PND. 2: 46,XX or AZFa or b microdeletion: TESE is contraindicated 3: Meiosis gene defects: considered TESE
CBAVD	*CFTR*	*ADGRG2*	Only if the partner has a *CFTR* mutation: consider PGD or PND
Oligospermia	Karyotyping	None	Chromosome rearrangement: considered PGD or PND
Macrozoospermia	*AURKC*	Whole-genome analysis	IVF is contraindicated for patients homozygote for a AURKC mutation or heterozygote composite
Globozoospermia	*DPY19L2*	Whole-genome analysis	None
Acephalic sperm	Whole-genome analysis	None	None
MMAF	Whole-genome analysis	None	None

Table 1: Genetic tests and their impact on clinical practice.

NOA: Non-obstructive azoospermia; CAVD: Congenital bilateral absence of vas deferent; MMAF: Multiple morphological abnormalities of the flagella; PGD: Preimplantation genetic diagnosis; PND: Prenatal diagnosis

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