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SHORT ARTICLE

Genetic testing in infertile couples

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Abstract

Approximately 15% of couples in western countries have infertility problems. Identification of genetic alterations responsible for infertility is important for therapy and to avoid transmission of genetic abnormalities that could impair the health of offspring, especially for couples with idiopathic infertility and those undergoing assisted reproductive techniques (ART).

The aim of this review is to summarize the main genetic tests to offer to infertile couples during diagnostic work-up and in cases of ART, considering future directions of risk assessment in the field of reproductive medicine.

Before offering a genetic test to an infertile couple, it is crucial to characterize their clinical and hormonal profile. Genetic testing should only be carried out when appropriate, that is when clinical and family history suggest a genetic cause of infertility. The genetic tests to offer to infertile couples must be targeted at infertility and should always consider the cost/benefit ratio. No causative genes have been identified for certain conditions, making clinical genetic testing impractical. Next generation sequencing (NGS) is a powerful tool for the identification of pathological mutations and for discovering new disease-associated loci in the field of reproduction. Comprehensive multigene panels for infertile risk assessment could simplify the diagnostic and therapeutic process. The main limitation is interpretation of the enormous amount of NGS data, since the clinical role and biological implications of variants, especially those of unknown significance, are still unclear.

Keywords: genetic testing, infertility, ART

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Introduction

In recent years, there has been great interest in the role played by genetic alterations in the pathogenesis of infertility of couples. Identification of a genetic cause of infertility is crucial for diagnostic work-up and in order to avoid transmission of genetic abnormalities that could impair the health of offspring of couples contemplating assisted reproductive techniques (ART). On the other side, the cost/benefit ratio should always be considered: genetic testing should only be carried out when appropriate, that is when clinical and family history suggest a possible genetic cause of infertility (1).

In this short review, we summarize the main genetic tests to propose to infertile couples during diagnostic work-up and in cases of ART, describing tests which are appropriate in cases of male and female infertility.

Genetic testing in male infertility

Before suggesting a genetic test for male infertility, the patient's condition should be characterized by collecting information on family and personal history, by evaluating clinical picture and by analyzing sperm count and hormonal data. Different genetic conditions can be responsible for different forms of male infertility. It is important to determine whether infertility is of testicular, pre-testicular or post-testicular origin.

Genetic testing in testicular infertility

Males with testicular infertility show sperm count evidence of azoospermia or oligozoospermia and high plasma concentrations of FSH and LH. In these cases, the first genetic test to perform is cytogenetic investigation. Simple karyotype analysis can detect most genetic causes of infertility of testicular origin. Several possible chromosomal abnormalities are:

- **47,XXY karyotype (Kinelfelter syndrome)** is the most common chromosomal abnormality in males (1:500-1:1000 newborns) and is responsible for about 25% of cases of non obstructive azoospermia (2). In some cases these patients have a 47,XXY/46,XY mosaic karyotype and residual spermatogenesis and can be enrolled in ART protocols, but risk generating 47,XXY or 47,XXX embryos.

- **46,XX karyotype** is detected in about 1 in 20,000 males, accounting for 2% of cases of azoospermia. In 90% of cases the condition is caused by abnormal cross-over of X and Y chromosomes during male meiosis in the patient's father, leading to translocation of the SRY gene, responsible for testis development, to the X chromosome (3). The lack of genes mapping to the long arm of the Y chromosome hampers sperm production, leading to infertility.

- **Structural aberrations of the Y chromosome** may be deletions of the long arm (Yq), isochromosomes for the short arm (Yp), ring Y chromosomes and translocations involving Yq. The rearranged Y chromosome is unstable during mitosis, and as a consequence patients often show somatic mosaicism 45,X/46,XY.

- **Reciprocal and Robertsonian chromosomal translocations involving autosomes** are frequently detected in the general population (1:600 and 1:1300, respectively). Both translocations can affect normal chromosome pairing during meiosis, inducing miscarriages or birth defects.

Patients with testicular infertility and normal karyotype can undergo molecular analysis for Yq microdeletions. The long arm of the Y chromosome contains several gene families involved in spermatogenesis, mapping to three loci: AZFa, AZFb and AZFc. Yq microdeletions are associated with azoospermia or severe oligozoospermia, accounting for about 10% of cases of male infertility (4). This abnormality is easily detected by a simple multiplex PCR or QF-PCR approach. This test should only be performed in patients with normal karyotype, and never in patients with normal sperm count.

Genetic testing in pre-testicular infertility

Pre-testicular forms of male infertility are characterized by low plasma concentrations of FSH and LH, and are less common than testicular forms. Two forms are Kallman syndrome and androgen insensitivity syndrome (AIS). Kallman syndrome is characterized by infertility and anosmia, and can be transmitted by X-linked inheritance or less often autosomal inheritance (3). In these cases it can be useful to analyze the KAL1 gene, responsible for the X-liked form. On the other hand, AIS is caused by mutations in the androgen receptor (AR) gene on the X chromosome, and comes in three forms: complete AIS (CAIS) characterized by a female external phenotype with intra-abdominal testis; partial AIS (PAIS) characterized by ambiguous genitalia, and mild AIS (MAIS) with infertility as the only clinical sign. MAIS accounts for about 2% of cases of azoospermia or severe oligozoospermia, and can be diagnosed by sequencing the complete coding region of the AR gene.

Genetic testing in post-testicular infertility

The major form of post-testicular infertility of genetic origin is obstructive azoospermia due to congenital bilateral absence of vas deferens (CBAVD). It is caused by mutations in the CFTR gene and is a less severe form of cystic fibrosis (CF). Thousands of different mutations are known in the CFTR gene; they can be classified as severe (CF) or mild (CF^m) mutations. CBAVD patients can also show a variant called "5T allele", which is never associated with classic CF. Two CF mutations are associated with classic CF, while a CF^m mutation combined with a 5T allele, even in compound heterozygosis with the CF mutation, causes less severe forms of the disease, including CBAVD. CBAVD patients can therefore have several different genotypes, namely: CF/CFm, CFm/CFm, CF/5T, CFm/5T or 5T/5T.

It is also necessary to calculate reproductive risk, since CBAVD patients can generate children by ART and transmit a mutant allele of *CFTR*. CBAVD patients always have two CFTR mutations, although first level analysis may only show one (5). Since the female partner of a CBAVD patient has a 1:25 *a priori* risk of being a carrier, it is necessary to know the exact genotype of both partners in order to calculate the risk for offspring (**Table 1**).

The identification of both CFTR mutations in CBAVD patients is therefore a prerequisite for correct genetic counseling. Screening of the most common CFTR mutations is not enough, since it generally detects CF but not CF^m mutations. CBAVD patients therefore require next generation sequencing as first-level test: the entire CFTR gene is sequenced.

Genetic testing in female infertility

Fewer forms of female infertility of genetic origin are known, but some must be investigated during diagnostic work-up of infertile couples.

Premature ovarian insufficiency (POI) can be observed in familial form in 10-15% of cases. In about 13% of cases of POI it is possible to detect numerical or structural abnormalities in the X chromosome. The most frequent is the 45,X karyotype, which in its classical form causes Turner syndrome, characterized by gonadal dysgenesia, amenorrhea and short stature. This condition is generally diagnosed at puberty and not during diagnostic work-up of infertile couples. However, in 40% of cases the karyotype occurs as a 45,X/46,XX mosaic,

Table 1. Possible phenotypes of offspring of CBAVD males and healthy female carriers of CF

Genotype of CBAVD patient	Genotype of female partner	Possible genotypes of offspring	Possible phenotypes of offspring
CF/CF^{m}	CF/wt	25% CF/wt 25% CF^{m}/wt 25% CF^{m}/CF 25% CF/CF	Healthy carrier Healthy carrier Atypical CF of variable severity Classical CF
CF/5T	CF/wt	25% CF/wt 25% 5T/wt 25% 5T/CF 25% CF/CF	Healthy carrier Healthy carrier Variable, normal to mild CF Classical CF
CF^{m}/CF^{m}	CF/wt	50% CF^{m}/wt 50% CF^{m}/CF	Healthy carrier Atypical CF of variable severity
CF^{m}/5T	CF/wt	25% CF^{m}/wt 25% 5T/wt 25% 5T/CF 25% CF^{m}/CF	Healthy carrier Healthy carrier Variable, normal to mild CF Atypical CF of variable severity
5T/5T	CF/wt	50% 5T/wt 50% 5T/CF	Healthy carrier Variable, normal to mild CF

and the patient can have an apparently normal menstrual cycle until manifestation of POI. Women with POI can also display structural abnormalities of the X chromosome, leading to loss of the entire short arm or involving the Xq26-Xqter (POF1) or Xq13.3-Xq21.1 (POF2) regions.

Premature ovarian insufficiency can also be associated with monogenic alterations, such as permutation of the FMR1 gene on Xq. This gene shows a variable number of CGG repeats in the promoter sequence. More than 200 repeats (full mutation) characterize Martin Bell syndrome, which comes with mental retardation, while 50-200 repeats are considered a "premutation", where gene function is spared but there is increasing risk of the full mutation in offspring. FMR1 premutations are found in about 3% of sporadic POI and up to 15% of familial forms. On the other hand, at least 13% of women carry an FMR1 premutation that will manifest POI during their life. Considering the risk of offspring with Martin Bell syndrome, it is recommended to analyze the FMR1 gene in POI patients, especially those combining POI and high serum concentrations of FSH (6).

Future directions of diagnostic work up

In spite of the great number of protocols available for the identification of causes of infertility, in about 30% of cases the etiology remains unknown. This gap may be filled by using the emerging technique of massive parallel deep sequencing, otherwise known as next generation sequencing (NGS). In order to explore the genetic basis of non-obstructive azoospermia (NOA), about 650 infertility-related genes from 757 NOA patients and 709 fertile males were sequenced using NGS. A significant excess of rare non-silent variants in genes that are key epigenetic regulators of spermatogenesis, such as BRWD1, DNMT1, DNMT3B, RNF17, UBR2, USP1 and USP26, were found in NOA patients (7).

Next generation sequencing with high sequence-tagged sites (STSs) was also used to obtain an overall picture of polymorphic deletions/microdeletions across the male-specific Y chromosome in 766 NOA patients and 683 matched controls, showing new Y-chromosomal microdeletions (8), different from the well-known AZFa,b,c microdeletions.

Five patients with total sperm immotility were genetically characterized by whole exome sequencing, identifying new DNA sequence variants in *CCDC103* and *INSL6* (9).

Next generation sequencing is therefore a powerful tool for identifying pathological mutations in genes known to underlie Mendelian disease, as well as for discovering new disease-associated loci and for comprehensive genetic assessment of male and female infertility.

Multigene panel kits for reproductive risk assessment were recently introduced (10). These kits are available on-line and can replace a variety of other assays for the genetic assessment of infertility in a single experiment. However, we still have much to learn regarding interpretation of the enormous amount of data issuing from massive NGS experiments, and specifically the clinical role and biological implications of variants, especially those of unknown significance. Genetic counseling is also imperative before and after genetic tests in order to make certain that patients are well-informed about possible implications, benefits and interpretations.

Conclusions

In conclusion, a comprehensive panel could simplify diagnosis and therapy, reducing turnaround time and lowering the overall cost of testing. However, for several conditions, no

causative genes have been identified and clinical genetic testing remains impractical. More collaboration is needed in this field to translate basic science into clinical practice.

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