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OPINION ARTICLE

Genetic screening for infertility: When should it be done?

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POF

Abstract Depending on the clinical findings, the infertile male patient needs genetic evaluation. Karyotype analysis and Y-chromosomal microdeletion screening should be performed in patients with azoospermia or severe oligozoospermia in order to rule out structural chromosomal abnormalities, Klinefelter syndrome and Y chromosome microdeletions. Infertile patients with obstructive azoospermia need cystic fibrosis transmembrane receptor gene screening, while in patients with hypogonadotropic hypogonadism mutation screening may be performed according to clinical features. All genetic analyses should be accompanied by expert counseling by a clinical genetist both in male and female patients.

Primary amenorrhea should be investigated by karyotype analysis and selected mutation screening according to the patient's clinical features. Karyotype analyses and *FMR1* gene screening is recommended in cases of POF. At present the infertility of patients with POF cannot be restored if the diagnosis is made after complete follicular depletion, but in some cases, early diagnosis by genetic investigation may instead lead to the advice of early conception or oocyte harvesting and preservation. In addition, the accumulation and annotation of array comparative genomic hybridization data might, in the near future, lead to the identification of pathogenetic copy number variations and genes involved in POF. Karyotype analysis of both partners is recommended in all couples with recurrent pregnancy loss. No routine genetic test can be recommended so far in patients with PCOS.

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1. Introduction

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Of all couples classified as infertile, female infertility accounts for about 40–50%. In 30–40% of infertile couples, male infertility is the cause, while the remaining 10–30% either is attributed to both male and female infertility or is unexplained (1) (Fig. 1).

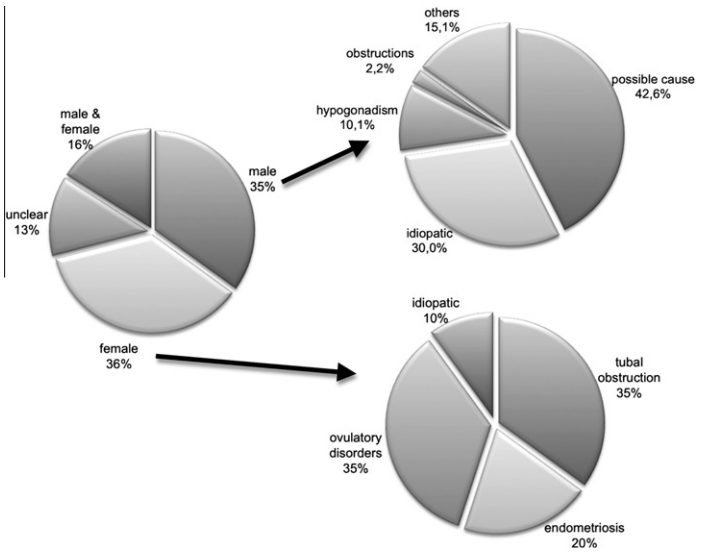

Figure 1 Causes of couple infertility. The male data are taken from Simoni et al. (2).

1.1. Male infertility

In the male the fertility criteria include: normal spermatogenesis, complete maturation of the spermatozoa during the passage through the accessory organs of the reproductive system, accessory organs' patency, production of an adequate volume of seminal fluid, ability to deposit the semen into the vagina, mobility of the spermatozoa adequate to reach the oocyte in the uterine tubes and penetrate it (3).

Currently, a discrete genetic cause can be demonstrated in over 20% of patients presenting with azoospermia and severe oligozoospermia who are obligatory candidates for genetic screening (own, unpublished data).

Among the best-known and most frequent genetic causes of male infertility are chromosomal abnormalities, mutations of the cystic fibrosis transmembrane receptor gene (*CFTR*) and Y chromosome microdeletions (4). Studies on families of infertile patients suggest that male infertility may have a familial component and an autosomal-recessive mechanism of inheritance was suggested (5,6).

The main indications for genetic testing in male infertility are azoospermia and severe oligozoospermia upon semen analysis (4). Even if detection of a genetic alteration will not substantially change the treatment, genetic testing should be performed for two reasons: to finalize a causal diagnosis; and to asses the genetic risk for the offspring in case of successful treatment. Expert counselling by a clinical geneticist should accompany every genetic test (4).

1.1.1. Chromosomal abnormalities

Numerical and structural chromosomal aberrations can be determined by karyotype analysis. The prevalence of chromosomal aberrations in infertile men is known to be around 10–15 times higher than in the general population, and ranges between 2% and 16% with an increasing frequency of abnormalities of autosomes with decreasing sperm count (Table 1) (8–10).

In the majority of cases, the infertile patient is an otherwise healthy male (apart from syndromic cases) and the chromosomal anomaly does not play any pathological role. However, men carrying a translocation, for example, have a markedly increased risk of inducing a pregnancy ending in miscarriage/stillbirth or fathering a child with varying degrees of mental and/or physical retardation in case of an unbalanced chromosome set of the offspring (4). Therefore, karyotype analysis is indicated in every infertile man with oligo- or azoospermia and, at least in cases of an abnormal karyotype, should be accompanied by genetic counselling.

1.1.2. Klinefelter syndrome

Men with Klinefelter syndrome (karyotype 47, XXY including mosaics) are common among infertile patients and may be identified by the following clinical features (11): reduced testicular volume (usually <6 bitesticular volume), azoospermia ($<8\%$ of Klinefelter patients have few sperm in their ejaculate), markedly increased gonadotropin levels (LH and FSH), low serum testosterone concentrations (<12 nmol/l), varying symptoms of hypogonadism (e.g., feminine body constitution and/or gynaecomastia may be present).

The vast majority of Klinefelter patients are infertile due to azoospermia. However, after the introduction of in vitro fertilization with intracytoplasmic sperm injection (ICSI), Klinefelter men have a chance to become fathers. Recent studies report high success rates of 30–70% for spermatozoa yields for ICSI by using testicular sperm extraction (TESE) with special microsurgical techniques locating focal spermatogenesis (12–17). Therefore, the possibility of testicular biopsy/TESE and cryopreservation of tissue should be discussed upon the first diagnosis of Klinefelter syndrome, even without an expressed wish for conception at the time (4).

1.1.3. Y-chromosomal microdeletions

Patients with non-obstructive azoospermia or severe oligozoospermia should be investigated for the occurrence of microdeletions of the Y chromosome, which represent one of the few well-recognized genetic causes of spermatogenetic failures resulting in male infertility (18). The breaking points of deletions are well-characterized and five main microdeletion

Table 1 Chromosomal abnormalities in infertile men (data taken from Mau-Holzmann (7)).

Abnormal karyotypes of 169 azoospermic males (originally analysed n = 1287)	
47, XXY	67%
Gonadal mosaicism	10%
46, XX	7%
Reciprocal translocations	4%
Robertsonian translocations	2%
Other gonosomal abnormalities	9%
Other autosomal abnormalities	1%
Abnormal karyotypes of 134 oligozoospermic males (originally analysed n = 1287)	
Robertsonian translocations	35%
Reciprocal translocations	16%
47, XXY	12%
47, XYY	8%
Inversions	8%
Extra structurally abnormal chromosomes	7%
Other autosomal abnormalities	1%
Other gonosomal abnormalities	13%

patterns have been identified: *AZF*a, P5-proximal P1 (*AZF*b), P5-distal P1 (*AZF*bc), P4-distal P1 (*AZF*bc) and b2/b4 (*AZF*c) (Fig. 2) (20). It is well-established that microdeletions of the Y chromosome occur in infertile but not in control men, although the frequency differs remarkably between countries, possibly depending on the selection criteria of the patients and on the ethnic background (18). Microdeletions of the Y chromosome are rare and the vast majority (< 80%) are deletions involving the *AZFc* region (4). In general, azoospermic men have a higher prevalence of microdeletions than oligozoospermic men (4). This is reflected by the semen parameters, which show azoospermia in about half of the patients, and only a few spermatozoa in the ejaculate occasionally present in the other half (4). Repeated semen analysis may be useful in such patients, as spermatozoa may occasionally appear in the ejaculate and be used for ICSI. Complete deletions of *AZFa* or *AZFb* are always associated with azoospermia. In general, TESE is possible in patients with *AZFc* deletions with a probability of recovering sperm of about 50%, but no sperm retrieval has been so far reported in patients with complete *AZFa* or *AZFb* deletions. Therefore, performing molecular genetic testing in these patients has a definite prognostic value for TESE. There are no clinical parameters beyond azoozpermia or severe oligozoospermia that can be used to predict the occurrence of a microdeletion of the Y chromosome (18). A progressive deterioration of spermatogenesis in patients with *AZFc* deletions has been proposed but never demonstrated (18,21).

1.1.4. Congenital bilateral absence of the vas deferens

Patients with obstructive azoospermia are candidates for genetic testing of mutations of the *CFTR* gene as they may have a congenital malformation of the Wolffian ducts, which are the precursors of the vas deferens, epididymis and seminal vesicles during foetal development (4). Thus, hypo- or aplasia of the epididymis or seminal vesicles can accompany the congenital absence of the vas deferens (CBAVD). In contrast to men with Y microdeletions, patients with CBAVD exhibit the distinct clinical features of azoospermia, decreased seminal volume, pH and markers of epididymal (α-glucosidase) and seminal vesicle (fructose) function in the presence of normal luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone levels.

In the majority of men with CBAVD, normal spermatogenesis will be found on testicular biopsy with histological evaluation/TESE, which also confirms the diagnosis of obstructive azoospermia, and thus these men have a high chance of fathering a child by ICSI (4). However, the risk of a child having cystic fibrosis (CF) is increased in comparison with the general population and can be estimated depending on the *CFTR* mutation(s) of the man and the carrier status of the female partner, as CF is inherited in an autosomal-recessive manner.

1.1.5. Hypogonadotropic hypogonadism

Hypogonadotropic hypogonadism (HH) is defined as a clinical syndrome characterized by low sex steroid and low gonadotropin levels resulting from a defect in the normal pulsatile secretion pattern of GnRH from the hypothalamus. Clinically, HH can be present with or without anosmia, the latter known as Kallmann's syndrome (22,23). Mutations of genes involved in the migration and/or function of the GnRH-secreting

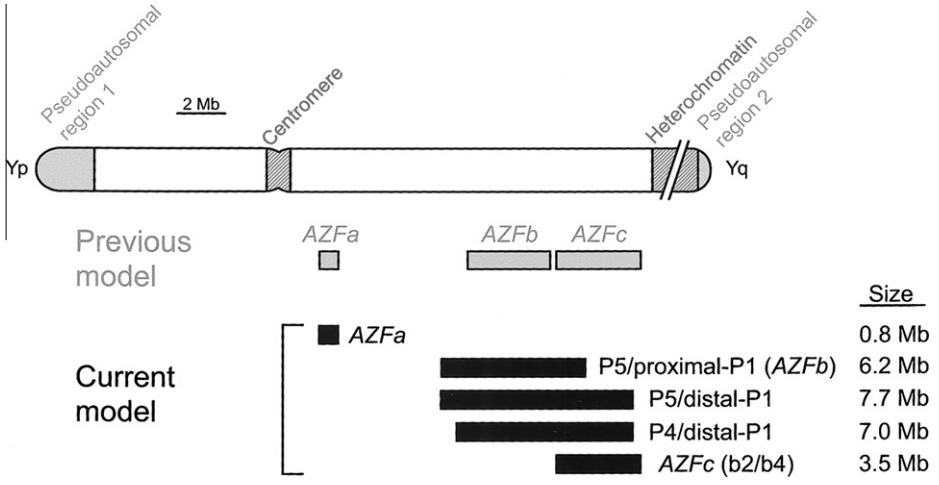

Figure 2 Current model of Y chromosome microdeletion pattern [from Repping et al. (19) with permission].

neurones are found in over 50% of the familial cases of HH and, more rarely, in sporadic cases (4). The first gene identified to be responsible for this phenotype was denominated *KAL1* and encodes for a protein of the extracellular matrix, *anosmin-1*. Successively, more genes were found to be mutated in HH with or without anosmia (Table 2).

The search for mutations in the genes involved in HH with or without anosmia requires direct gene sequencing or sequencing after mutation screening by melting curve technology or an equivalent method (4). As these techniques are demanding in terms of time and resources, mutational analysis should be performed after careful genetic counselling, which allows full definition of the symptoms, identification of the familial cases and definition of the pattern of inheritance (autosomal-dominant, autosomal-recessive or X-linked), thereby limiting the number of genes to be screened.

1.1.6. Idiopathic infertility

Chromosomal anomalies are found in about 7% men with idiopathic spermatogenic failure, predominantly numerical/structural in azoospermic men and translocations/inversions in oligospermic men (24). Routine karyotyping of men with sperm densities less than 10 million/ml, even in the absence of other clinical presentations, is recommended because infertility is associated with higher rates of aneuploidy in ejaculated or testicular sperm and increased chromosomal defects in ICSI offspring (24). When AZF-deleted sperm are available and used for ICSI, fertility defects on male offspring seem inevitable (24). Genetic causes of idiopathic infertility must be sought by systematic valuation of infertile men and affected couples must be informed about the implications of such diagnosis for assisted reproductive technology outcome and their potential offspring. There is currently no indication for individual gene analysis in idiopathic infertility (4).

1.2. Female infertility

Some major causes underlying female infertility are polycystic ovary syndrome (PCOS), premature ovarian failure (POF) and recurrent pregnancy loss (RPL).

1.2.1. Polycystic ovary syndrome

According to the Rotterdam criteria two of the following have to be met in order to fit the definition of PCOS: chronic oligo-/anovulation, clinical and/or biochemical evidence of hyperandrogenism, and polycystic ovaries (25). As the PCOS consists in a set of symptoms, biochemical features, and signs that can occur in various combinations, it can also be associated with features of the metabolic syndrome including insulin resistance and obesity, with a 7-fold increased risk of developing type 2 diabetes (26). As a result of these possible associations the clinical expression of the syndrome varies.

Several genes controlling ovarian function and metabolism are associated with increased susceptibility to PCOS, but none is strong enough to correlate alone with susceptibility to the disease, or response to therapy. A large number of studies have investigated polymorphisms in the gene encoding the receptor for FSH (*FSHR*). Only one single nucleotide polymorphism (SNP) in exon 10 of the *FSHR* gene, *FSHR p.N680S*, was consistently identified as having a significant association with ovarian response to FSH, but it does not seem to play any major role in the PCOS (27). The amount of FSH needed for controlled ovarian hyperstimulation to achieve similar peek estradiol levels was significantly lower in women with the N/N genotype at position 680 of the *FSHR* gene compared with women carrying the S/S genotype or N/S genotype, indicating lower ovarian sensitivity to FSH in vivo for the S680 allele (28).

Both inactivating and activating *FSHR* mutations may lead to relevant clinical effects. Inactivating mutations can result in primary or secondary amenorrhea, infertility and premature ovarian failure (POF), while activating mutations can predispose to ovarian hyperstimulation syndrome (OHSS) as a consequence of exogenous FSH administration or even with a spontaneous onset (29). If the effects of *FSHR* gene mutations and polymorphisms on reproductive pathologies are studied further, interesting scenarios for the clinical application of gene sequencing in well-defined conditions could be opened in the near future. Two examples thereof are the precocious identification of women at risk of POF and the possibility of "tailoring" controlled ovarian stimulation in IVF with an FSH stimulation schedule based on the individual responsiveness of *FSHR* (29). Besides the influence of *FSHR* genotype on ovarian responsiveness, other genetic factors have been analysed and reported to be involved in modulating the ovarian sensitivity to FSH. Among candidate genes we can mention the *ER1* (30–32) and *ER2* genes (32), as well as the anti-Müllerian hormone (*AMH*) and AMH type II receptor genes (33) and the *MTHFR* (methylenetetrahydrofolate reductase)

Table 2 Genes mutated in Isolated Hypogonadotropic Hypogonadism and in Kallmann syndrome (4).

Acronym	Name	Location	Gene ID	Function
GNRHR	Gonadotropin-releasing hormone receptor	4q21.2	2798	Receptor for the gonadotropin-releasing hormone
KISS1	KiSS-1 metastasis-suppressor (metastin)	1q32	3814	Ligand of GPR54: stimulation of GnRH secretion
GPR54	G protein-coupled receptor 54	19p13.3	84634	Receptor for Kiss-1: stimulation of GnRH secretion
KAL1	Kallmann syndrome 1 sequence (anosmin-1)	Xp22.32	3730	Possible function in neural cell adhesion and axonal migration
FGFR1	Fibroblast growth factor receptor 1	8p11.2–p11.1	2260	Binds both acidic and basic fibroblast growth factors
FGF8	Ffibroblast growth factor 8	10q24	2253	Member of the fibroblast growth factor (FGF) family involved in organogenesis
PRKR2	Prokineticin receptor 2	20p12.3	128674	G protein-coupled receptor for prokineticins
PRK2	Prokineticin 2	3p13	60675	Chemoattractant for neuronal precursor cells in the olfactory bulb
CHD7	Chromodomain helicase DNA binding protein 7	8q12.2	55636	Expressed in undifferentiated neuroepithelium and in mesenchyme of neural crest origin
GnRH1	Gonadotropin-releasing hormone	8p21 p11.2	2796	Stimulation of LH and FSH secretion

(34,35) gene. Various studies have analysed the effects of polymorphisms in genes encoding for metabolic enzymes on ovarian response such as the *CYP19A1* gene and the *BMP15* gene. As ovarian responsiveness to FSH may be a polygenic trait, the combined role of all these factors needs further investigation through large number and well-designed studies on patients undergoing ovarian hyperstimulation before drawing conclusions about the effects of these various genotypes on the ovarian response to the FSH (27).

1.2.2. Recurrent pregnancy loss

RPL, occurring in 0.5–1% of total pregnancies, is defined as three or more consecutive spontaneous abortions before 20 weeks of gestation. Although an immunology-based aetiology underlying unexplained RPL has been demonstrated, the exact molecular and genetic mechanisms are still poorly understood. Recent studies showed that particular immunological abnormalities and thrombophilic aberration might underlie RPL. Approximately 25–51% of miscarriages in women with RPL are associated with a chromosomal abnormality in the conceptus (36–39), and karyotyping of abortuses should be performed to determine whether there is a cytogenetic reason for the loss (39).

Based on equal frequencies of abnormal test results found among patients with two, three or four or more recurrent pregnancy losses, Jaslow et al. (40) suggests that a complete evaluation of evidence-based factors, i.e., parental karyotyping, uterine anatomy, lupus anticoagulant, anticardiolipin antibodies, factor V Leiden, and TSH should be recommended to all couples with two or more consecutive pregnancy losses (40). Caution must be exerted in the interpretation of an abnormal screening result, as the factor identified may not be the causative factor for pregnancy loss.

1.2.3. Premature ovarian failure

POF is another condition thought to be genetically determined. It is defined as a primary ovarian defect characterized by absent menarche (primary amenorrhea) or premature depletion of ovarian follicles/arrested folliculogenesis before the age of 40 years (secondary amenorrhea) (41,42).

The causes of POF are extremely heterogeneous. Karyotype analyses should be performed in primary amenorrhea and in cases of POF at very young age. Mutations of several genes effecting hormone action and follicle function (*FSH*, *FSHR*, *LH*, *LHR*, *CYP17*, *CYP19*, *BMP15*, *GDF9*, and *GPR3*) have been occasionally found in humans, but none are common. Other genes such as DNA binding proteins and transcription factors like *NOBOX* and *LHX8*, and RNA binding proteins like *NANOS* are involved in oogenesis. Plausible causative mutations have been identified in a few women (*NOBOX*, *GDF9*, and *LHX8*), but are exceedingly rare (only 1–2% of cases). Thus, considerable heterogeneity exists in POF and no routine genetic screening can be recommended so far beyond karyotype. Two of the candidate genes are located on the X chromosome. *FMR1* gene (Xq27.3) mutations or premutations are typically associated with secondary amenorrhea in female relatives of male patients with mental retardation (43). A significant risk of POF is represented by a premutation of fewer than 100 CGG repeats in female carriers of fragile X syndrome (44). *BMP15* gene (Xq11.2) defect has so far been described in two sisters with primary amenorrhea and heterozygous for the mutation. This defect would represent an unusual example of an X-linked disease in which affected females inherit the mutation from their unaffected father (45) but has been questioned by others (46).

An opportunity to predict the likelihood of early menopause could be offered by an early diagnosis of familial POF, which could also allow considering different reproductive choices, such as freezing embryos or having children earlier. It is important for clinicians to make a timely diagnosis and begin appropriate strategies for symptom management, emotional support, and risk reduction, because POF has cumulative negative effects over time (47). Karyotype evaluation and other cytogenetic investigations are useful to identify major X chromosome abnormalities.

Recently, Aboura et al. (48) identified 31 copy number variations (CNVs), using array comparative genomic hybridization (a-CGH) analysis in a cohort of patients with POF. Three CNVs were identified on the X chromosome and 28 on autosomal chromosomes. Eight of thirty-one CNVs reported resulted statistically significant although no correlation could be made between the CNVs and the phenotype of POF.

Genetic counselling is recommended for several reasons, when a genetic form of POF is suspected or identified. Counselling is of particular importance in cases of families with X-linked mental retardation (Fragile X syndrome). The expansion of CGG repeats is associated with gene silencing resulting in male mental retardation and in POF with secondary amenorrhea in female carriers (44). When a female is born from a family with other female members affected with POF, genetic investigations may be useful for the early diagnosis of genetic defects underlying POF. Pedigree studies on affected families showed a mode of inheritance suggestive of autosomal dominant sex-limited transmission or X-linked inheritance with incomplete penetrance (47).

2. Conclusions

Depending on the clinical findings, the infertile male patient needs genetic evaluation. Karyotype analysis and Y-chromosomal microdeletion screening should be performed in patients with azoospermia or severe oligozoospermia in order to rule out structural chromosomal abnormalities, Klinefelter syndrome and Y chromosome microdeletions. Infertile patients with obstructive azoospermia need *CFTR* gene screening, while in patients with HH mutation screening may be performed according to clinical features. All genetic analysis should be accompanied by expert counseling by a clinical genetist both in male and female patients (4).

Primary amenorrhea should be investigated by karyotype analysis and selected mutation screening according to the patient's clinical features. Karyotype analysis and *FMR1* gene screening is recommended in cases of POF. At present the infertility of patients with POF cannot be restored if the diagnosis is made after complete follicular depletion, but in some cases, early diagnosis by genetic investigation may instead lead to the advice of early conception or oocyte harvesting and preservation. In addition, the accumulation and annotation of array CGH data might, in the near future, lead to the identification of pathogenetic CNVs and genes involved in POF (48). Karyotype analysis of both partners is recommended in all couples with RPL (49). No routine genetic test can be recommended so far in patients with PCOS.

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