Genetic testing in couples with infertility

Rolf-Dieter Wegner[1], Matthias Bloechle[2]

[1] Zentrum für Pränataldiagnostik, Kudamm 199 und Institut für Humangenetik, Charité Campus Virchow, Berlin

[2] Kinderwunschzentrum an der Gedächtniskirche, Berlin

Reviewers: Moritz Meins, Göttingen and Lutz Pfeiffer, Berlin

Summary

Genetic changes are frequent causes of fertility problems. Genetic counseling combined with chromosome analysis is indicated in infertile or subfertile couples. In case of a normal karyotype, molecular diagnostics can be performed owing to the clinical picture. Premature ovarian failure might be caused by premutations in the FMR1 gene, while polycystic ovaries combined with other symptoms of late-onset AGS result from mutations in the CYP21A2 gene. In azoospermic or oligospermic men without chromosomal abnormalities, analyses of the CFTR gene and the AZF region are indicated. According to the forthcoming "Gendiagnostikgesetz" In Germany – a law regulating genetic diagnostics – thorough counseling prior to diagnostic procedures will be compulsory in certain cases. Genetic counseling has got to be provided in the case of pathological findings. The great number of rare genetic causes of infertility is best disclosed in the course of genetic counseling combined with a clinical examination.

Introduction

Worldwide, the prevalence of couples who remain involuntarily childless over a period of twelve months ranges from 3,5% to 16,7% with a median of 9%. In the Federal Republic of Germany, the prevalence in 2001 ranged from 5% to 9% with a remarkable difference between the old and new states of Germany. Approximately 3% of all relationships remain permanently childless.

According to the Federal Statistical Office, the reasons for involuntary childlessness in patients seeking assisted reproductive technology can be attributed to male infertility in 11%, to female infertility in 24% and to both partners in 40%. However, in 25% of cases, the reason for infertility remains unidentified. Genetic testing is of special importance concerning treatment options and preventative measures for the patient as well as for the offspring, for instance in the case of FMR1 gene mutations. Genetic factors include chromosomal abnormalities, single gene mutations and monogenic or multifactorial syndromes and diseases.
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The diagnostic measures in couples that are involuntarily childless are usually initiated by the gynecologist, andrological urologist, the dermatologist or the human geneticist. Therapy is predominantly performed at specialized centers of assisted reproduction, as in 50% of cases, medical treatment of childlessness results in assisted reproduction.

This article presents both common genetic modifications as well as genetic testing in involuntary childless couples. The article is divided into four sections: general considerations on genetic testing, genetic testing in female infertility, genetic testing in male infertility and genetic testing in cases of spontaneous abortions. Genetic testing is only one component of an extensive diagnostic process, thus, recommendations and guidelines to the matter as well as to legal matters are included in the following chapter, followed by a short chapter on terms and definitions. Preimplantation Genetic Diagnosis (PGD) will not be dealt with here.

Recommendations, guidelines and regulations

In various publications on the diagnostic and therapeutic procedures in couples with infertility (the ESHRE Capri Workshop Group 2000; Jantke 2005), in male infertility (Ochsendorf et al. 2002; Jungwirth and Dunzinger 2003), in female infertility (ESHRE Capri Workshop Group 2008) as well as in spontaneous abortions, (Wieacker et al. 2005; Steck 2006; DGGG 2008), genetic testing after adequate consultation is consistently recommended or even demanded in couples with unexplained infertility. The guidelines of the German Medical Association on assisted reproduction (2006) request genetic counseling in fertility disorders as well as in recurrent abortions or stillbirths. Genetic counseling, focusing on an extensive evaluation of the familial history and, if necessary, clinical-genetic examinations, is required in order to decide whether and which further genetic testing is appropriate for the couple. The disclosure of a pathological finding always has to be carried out in person.

Detailed information on the diagnosis of genetic factors have been published by the German Society of Human Genetics (GfH) as well as from specialists groups and are available for the CFTR gene (Ludwig et al. 2004; GfH 2009a; Els Dequeker et al. 2009), the FMR1 gene (GfH 2009b; Joint SOGC-CCMG Meeting 2008) as well as for Y chromosome microdeletions (Ludwig et al. 2004; Simoni et al. 2004; GfH 2007).

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Soon, the "Gendiagnostikgesetz" (gene diagnostics law), adopted by the German Bundestag, will come into effect. It requires profound medical counseling with documentation and written consent of the patient prior to genetic testing. Genetic counseling can be offered in the case of negative results but is obligatory in the case of pathological results. Highest standards have to be met in predictive diagnostics. Here, counseling by a human geneticist or a doctor with the appropriate education is essential.

Definitions

In the present article, infertility/subfertility is defined as the inability of a couple to conceive a child for at least one year albeit explicit will and regular, unprotected sexual intercourse. In contrast to the WHO criteria, which define the period as two years, one year is more appropriate due to time pressure and increasing age of the patients.

The term subfertility is used if there is a remaining chance for natural conception and childbirth, for example in patients with abortions or in patients with oligozoospermia. Infertility is present if pregnancy can only be achieved by assisted reproduction or if the patient is entirely impotent.

General considerations regarding genetic testing

Table 1: Various methodological approaches of genetic analyses with their respective resolution in routine diagnostics and fields of application of light microscopy

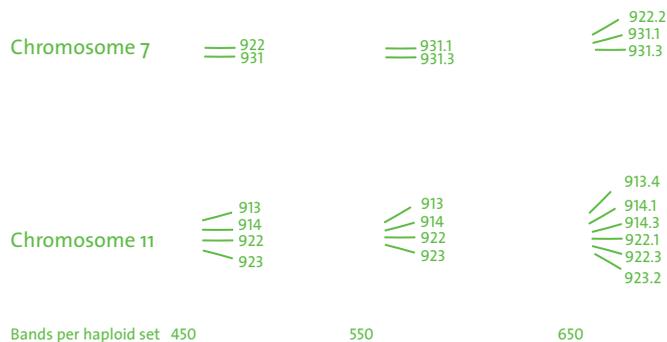| Genetic analysis	Chromosomal abnormality detectable with light microscopy	Resolution (DNA base pairs)
Cytogenetic	yes	≥ 5 mb
Molecular cytogenetics	yes, indirect diagnostics (fluorescence microscopy)	≥ 1 kb
Molecular	no	≥ 1 bp

Figure 1: Giemsa banding ("GTG" banding) of the chromosome pairs 7 and 11 with depiction of increasing resolution

Depending on the existing problem, genetic testing is performed with one or more of the three following methodological approaches: chromosome analysis, molecular cytogenetics (fluorescence in situ hybridization, FISH) or molecular analysis of DNA (Table 1). Chromosome analysis provides an overview of all chromosomes, which is sufficient for the determination of aneuploidy. Aneuploidy is defined as a numerical alteration from the usual chromosomal set of 46 chromosomes, such as the Klinefelter syndrome or the Turner syndrome. It also enables the detection of structural alterations, such as translocations, which are a common cause for abortions. However, the detection of structural alterations is limited by microscopic resolution. As an indicator of quality, the number of chromosome banding per haploid set is used (Fig.1). For instance, the cytogenetic guidelines from the German Society of Human Genetics (GfH) request high chromosomal banding resolution of at least 550 bands per haploid set for couples with recurrent abortions. The resolution has to be specified in the cytogenetic reports. Higher resolutions in the submicroscopic range enable molecular cytogenetic testing. However, this technique is only occasionally utilized in fertility diagnostics, examples are the more detailed characterization of chromosome translocations or the characterization of Y chromosomal abnormalities.

High-resolution diagnostics are required for gene mutations such as deletions in the azoospermia factor (AZF) region or in FMR1 gene mutations.

Up to now, approximately 300 gene mutations, of which 70 are syndromes, are known to cause reproductive disorders. Thus, the present article only focuses on the most common genetic causes of infertility.

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                         │Chromosome analysis│
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Male / Female

Genetic counseling

Chromosome analysis

Numerical alteration, e.g. 47, XXY: Klinefelter syndrome

Numerical alterations, e.g. 45, X: Tumor syndrome

Positive findings

Positive findings

Structural alterations, e.g. translocation: abortions optional: **FISH analysis**

Structural alterations, e.g. translocation: abortions optional: **FISH analysis**

Negative findings

Molecular analysis

Non-obstructive azoospermia, severe to moderate oligozoospermia
→ **AZF screening program**

Premature ovarian failure Hypergonadotropic ovarian insufficiency, premature menopause:
→**FMR1 gene analysis**

Obstructive azzospermia, congenital bilateral aplasia of the vas deferens or severe oligozoospermia, aplasia of the vas deferens:
→**CFTR analysis**

Abortions:
→ **thrombophilia diagnostics**
Factor-II- and factor-V(MTHFR)-gene

Search for other gene mutations or syndromal causes:
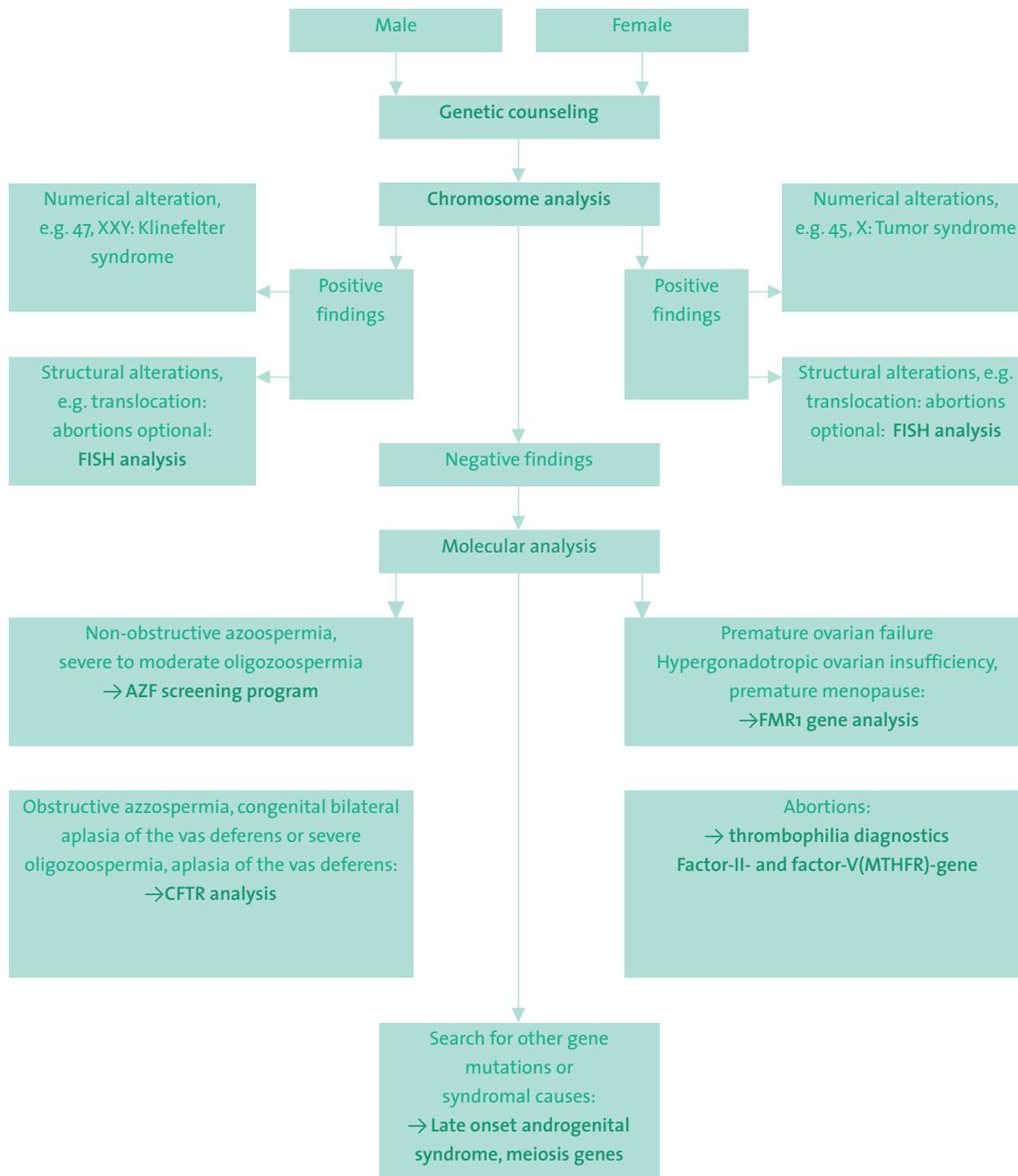→ **Late onset androgenital syndrome, meiosis genes**

Figure 2: Procedure of genetic testing in involuntarily childless couples

The procedure of routine genetic testing is depicted in Figure 2.

Several scientific studies have consistently shown that infertile couples have an increased frequency of chromosomal anomalies in both partners, independent of the cause of infertility. Genetic testing should thus always begin with a classical chromosome analysis. For this purpose, a whole blood sample of at least 2 ml diluted with heparin is required. For an optional molecular genetic analysis, a whole blood sample of at least 5 ml supplemented with EDTA is necessary.

Genetic testing in female infertility

From all infertility cases, 3,3% to 9,8% are caused by female chromosomopathies. If the karyotype is normal, molecular genetic analyses, especially in order to test for FMR1 gene mutations in premature ovarian failure and CYP21A2 mutations in polycystic ovaries and/or other symptoms of late-onset androgenital syndrome (21-hydroxylase deficiency), have to be considered.

According to the new "Gendiagnostikgesetz" in Germany, genetic counseling with clinical genetic diagnostics will have to be carried out prior to genetic testing. This shall provide thorough information of the patient regarding the background of the testing and the possible consequences for the carriers and for potentially affected family members. A highly relevant example for this is the risk for fragile X premutation carriers to develop late-onset neurodegenerative diseases (see below in molecular genetic diagnostics). The patient presents only with subfertility, however, genetic testing also includes predictive diagnostics. Predictive diagnosis is limited to specialists, for example a human geneticist or a doctor with a comparable qualification in the field. Simultaneously with taking the familial history, the existence of syndromal genetic diseases or multifactorial features should be assessed.

Chromosome analyses

Aneuploidy

Turner syndrome
The karyotype 45,X, which causes Turner syndrome, is a common chromosomal abnormality in females. It affects 1/2500 live-born girls. Diagnosis is often delayed until the first years of school when growth retardation becomes apparent. Some are only diagnosed when presenting with primary ovarian failure, mostly as primary amenorrhea. The patient's intelligence is normal.

An important aspect for prognosis and therapeutic options is the existence of genetic mosaicism. Mosaicism is defined as the presence of at least two cell lines with different karyotypes, which have developed from a single fertilized egg. The high incidence of mosaicisms in Turner syndrome of 50% is quite remarkable. Thus, apart from 45,X cell lines, also cell lines with structural alterations of the X chromosome (39%), the Y chromosome (6%) and numerical alterations (7%) can occur. In some mosaicisms, for example mos 45,X/46,XX, germ cell formation is functional, which allows for a normal or only slightly impaired fertility. If the chromosomopathie is detected early, the patient should be informed about the high risk of premature ovarian failure and thus be recommended to procreate early in life. Pregnant women with Turner syndrome or mosaicism have an empirical chance of 15% to have children with 45,X monosomy.

A mosaicism mos 45,X/46,XY is associated with a high risk for gonadoblastoma, thus, gonadectomy should be considered.

The 47,XXX karyotype has an incidence of 1 in 1000. Two thirds of the carriers have a clinically normal phenotype. One third has learning difficulties and psychotic disorders. Premature ovarian failure with infertility has been described for carriers of this chromosomal abnormality. In women with XXX karyotype, the risk for the offspring to carry X chromosomal aneuploidy is < 1%.

Structural chromosomal alterations
The structural chromosomal abnormalities include translocations, deletions and duplications. The most common structural alterations are translocations, which are further subdivided into balanced (chromosomal exchange without loss or gain of genetic material) and unbalanced translocations (chromosomal exchange with loss or gain of genetic material). Balanced translocations involving two autosomes occur in 3.35‰ of all newborns. These are further distinguished into:
1. Robertsonian translocations, in which two acrocentric chromosomes fuse (incidence 1.35‰) (Fig. 3) and
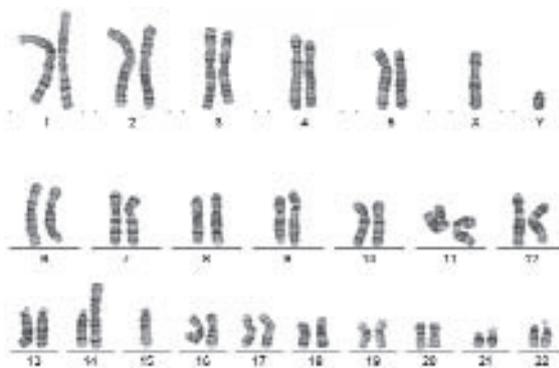2. Classical balanced translocations (incidence 2.0‰) (Fig. 4).

Figure 3: Male karyogram with balanced Robertsonian translocation of chromosomes 14 and 15: 46,XY,rob(14;15)
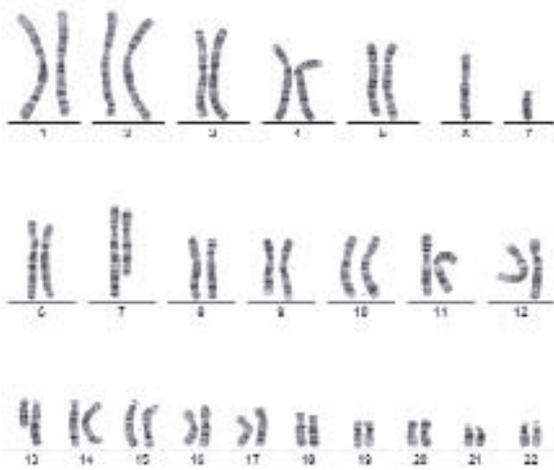
Figure 4: Male karyogram with balanced translocation of chromosomes 7 and 13: 46,XY,t(7;13)(q36;q14.3)

Balanced translocations, hampering the normal course of meiosis, are increasingly found in the patient population with infertility or subfertility. In balanced translocations, the chromosomes form translocation quadrivalents instead of bivalents, which can cause chromosomal segregation problems and germ cell arrest. Amongst all infertility patients, females are less affected by translocations than males. Translocations can very often lead to abortions due to chromosomal imbalances (see below).

Structural alterations involving the X chromosome increase the risk for premature ovarian failure and infertility.

The loss (deletion) of the short arm of the X chromosome results in the typical clinical picture of Turner syndrome. The chromosomal findings can vary from a simple loss of the short arm (monosomy Xp) to its partial loss or the loss of the short arms due to the fusion of the long arms of the X chromosome at the centromere. The latter results in the karyotype of monosomy Xp combined with trisomy Xq (Isochromosom Xq).

Molecular genetic testing

FMR1 gene (Fragile X mental retardation 1)

Mutations in the FMR1 gene located in the long arm of the X chromosome (Xq27.3) are differentiated into full mutations, premutations and intermediate alleles. Full mutations in the male cause the fragile X syndrome (FXS) with mental retardation and other associated symptoms. In the female, the risk for learning difficulties or minor mental disability is increased. Premutations in the female correlate with infertility as well as with an increased risk for late-onset progressive neurodegenerative diseases.
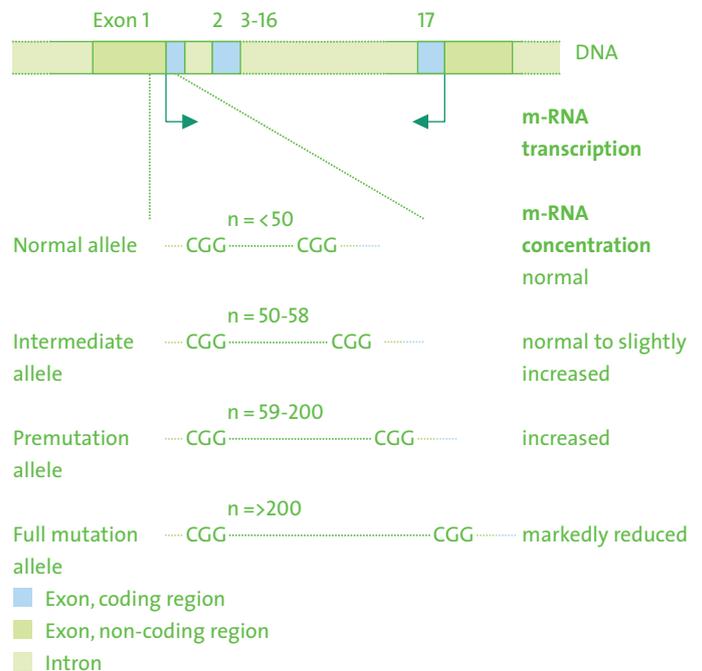

Figure 5: Structure of the FMR1 gene displaying the transcription sites as well as the various CGG repeats and their repercussions on the transcription rate.

A typical FMR1 gene mutation is caused by expanded CGG repeats in the untranslated region of the first exon (Fig. 5). Normal alleles have 5-49 repeats, intermediate alleles 50-58, premutations 59-200 and full mutations > 200. An intermediate allele has a low risk of expansion (~ 6.6%) and its expansion to a full mutation has not been described yet. A full mutation develops due to transmission of a premutation from the mother with the subsequent expansion of CGG repeats. A full mutation results in the hypermethylation of the surrounding DNA region with a more or less complete transcriptional gene silencing and thus a missing gene product. In contrast, premutations result in an overexpression of the gene.

Although the prevalence of full mutations is rather low with 1:8000 in women, the premutation rate varies from 1:75 to 1:152, depending on the geographical region. 0.8% to 7.5% of patients with sporadic premature ovarian failure are carriers of premutations and amongst patients with hereditary premature ovarian failure, the rate is 13%. In another survey, the premutation prevalence was 13% to 26%. On average, these patients enter menopause five years earlier compared to the general population. Cognitive abilities correspond to that of the control group. It should be emphasized that carriers of premutations have an increased risk for male offspring with fragile X syndrome and an increased risk for female progeny with learning difficulties or slight mental impairment.

Women with fertility issues and increased FSH levels before reaching the age of 40 as well as women with premature ovarian failure/menopause in two family members but with a normal chromosomal set should undergo genetic counseling and molecular genetic testing of the FMR1 gene.

21-hydroxylase deficiency (CYP21A2 gene) – adrenogenital syndrome

21-hydroxylase deficiency by a gene mutation can be a monogenic cause of infertility and often results is polycystic ovary syndrome in women. Approximately 95% of all androgenital syndrome cases are caused by the autosomal recessive transmission of 21-hydroxylase deficiency caused by a mutation in the CYP21A2 gene located on chromosome 6. The disease is further classified into the classic, severe type (two severe mutations) and the nonclassic type – also known as late-onset androgenital syndrome. The latter comprises either two alleles with mild mutations or compound heterozygosity (two alleles with different mutations of the CYP21A2 gene) with one allele carrying a mild and the other a severe mutation. The prevalence of the classic type is 1:10000 to 1:15000. Late-onset androgenital syndrome with usually > 20% enzymatic activity has a prevalence of 1 in 1000.

In the context of subfertility/infertility of the female, late onset androgenital syndrome is of importance, as the classic form can already be diagnosed in child or infancy stages (newborn screening in Germany). Apart from polycystic ovaries and menstrual disorders, secondary amenorrhea or oligomenorrhea, hirsutism and acne as well as increased DHEAS and 17-OH progesterone blood levels in the follicular phase of the cycle are typical symptoms of late-onset androgenital syndrome. Where there is reason to suspect late-onset androgenital syndrome, molecular diagnostics should be performed in order to exclude or confirm mutations in the CYP21A2 gene.

If the patient is found to be homozygous or is a compound heterozygote, the partner should be tested, as the heterozygote frequency in central Europe is 1 in 50 and thus, the possibility for the offspring to carry two mutated alleles is 1%.

Other mutations and syndromes

There are a number of other genetic modifications that are associated with syndromal or non-syndromal ovarian failure, XX gonadal dysgenesis or other fertility issues. For instance, mutations in the WT1 gene cause Denys-Drash syndrome, which goes along with kidney malformations. In the case of normal cytogenetics, the presentation and diagnosis of these patients in the course of genetic counseling is highly indicated.

Genetic testing in male infertility

Amongst all infertile men, genetic factors account for 30% of the cases, although only a small proportion of these factors can be tested for. The frequency of chromosomal abnormalities is 10 to 15 times higher than in the general population. There is a clear correlation between sperm number and chromosomal abnormality rate. Thus, the prevalence of chromosomal abnormality is found to be 1.7% to 3.5% in men with ≤20 million spermatozoa/ml, 6.5% in men with ffi10 million spermatozoa/ml and 8% in men with ≤1 million spermatozoa/ml. However, it is not appropriate to define a certain sperm number as a threshold value for the indication of cytogenetic analysis, as also subfertile men with more than 50 million spermatozoa/ml have an increased rate of chromosomal abnormalities.

In men with oligoospermia or azoospermia, molecular genetic testing confirms a high frequency of deletions in the long arm of the Y chromosome (Yq11.21-23) encompassing the azoospermia factor (AZF) region as well as specific mutations in the CFTR gene.

Chromosome analyses

Aneuploidy

In the male, Klinefelter syndrome (47,XXY) represents the most common aneuploidy with an incidence of 1:600 to 1:800 in all male newborns. Key symptoms include reduced testicular volume, hypergonadotropic hypogonadism and azoospermia. The chromosomal abnormality is not associated with mental disabilities, however, carriers often present with behavioral problems. There is an increased risk for osteoporosis and breast cancer. Hormone substitution is indicated.

The clinical heterogeneity is remarkable, leading to the fact that an estimated 74% of all men with XXY karyotype are not diagnosed (Abramsky and Chapple 1997).

Progeny of XXY men by ICSI (intracytoplasmic sperm injection) after TESE (testicular sperm extraction) have been described. They have an increased risk for gonosomal aberrations.

There is cytogenetic heterogeneity in the Klinefelter syndrome mosaicisms, for example the karyotype mos 47,XXY/46,XY or mos 47,XXY/45,X. The clinical manifestations of the former are milder compared to the pure XXY karyotype. For instance, spermiogenesis has been described for carriers. Carriers of mosaicisms with male and female chromosomal sets and dysgenetic gonads have a higher risk for gonadoblastomas.
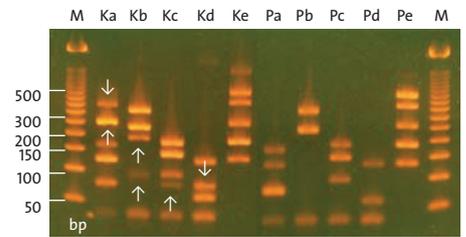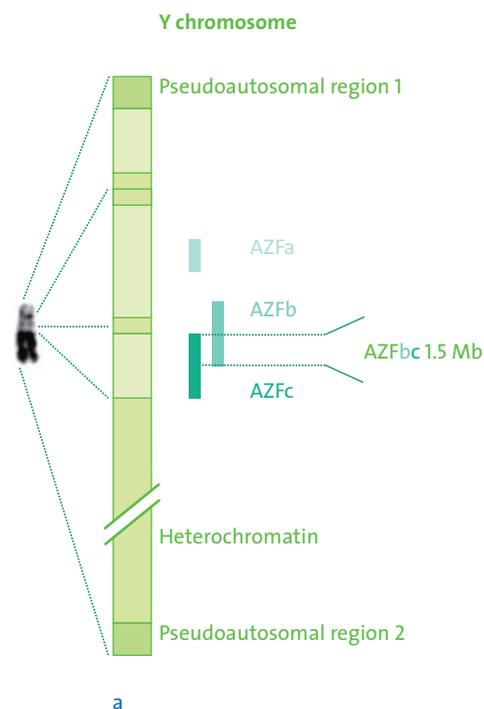
Klinefelter syndrome variants include karyotypes of 48,XXYY and 48,XXXY. In most cases, the manifestations are severe and include azoospermia, mental retardation and behavioral problems.

Structural chromosomal alterations

Balanced autosomal translocations are the most common structural chromosomal abnormalities (see above). Clinically relevant deletions of the Y chromosome, the XX karyotype in the male, the Y isochromosome, and an additional marker chromosome are only rarely found in infertility.

Molecular genetic testing

Azoospermia factor

Y chromosome

Pseudoautosomal region 1

AZFa

AZFb

AZFbc 1.5 Mb

AZFc

Heterochromatin

Pseudoautosomal region 2

a

b

Figure 6:

a: Y chromosome with C band staining (CBG-bands) and a schematic representation of the Y chromosome with the position of the AZF intervals a, b and c

b: analysis of the AZF region of the Y chromosome for the confirmation of the presence or absence of 20 selected regions with PCR based technology (Promega). 5 PCRs are performed simultaneously (Multiplex Master Mix), in order to cover the complete AZF region. Amplification of the genomic DNA of these patients (Pa to Pe) and of healthy men (positive control Ka to Ke) was performed for each of the five Multiplex-Master-Mix sets. The patient has a deletion in the AFZc region and the distal AZFb/c region. The deleted bands are highlighted with an arrow in the positive control. Lane M: marker DNA for size determination of the DNA fragments.

In patients with non-obstructive azoospermia or oligozoospermia, there is an increased incidence of deletions in the long arm of the Y chromosome, region Yq11.21-23. In this region, genes involved in meiosis are present in high density and arranged in a complex manner. This region is also known as the azoospermia factor (AZF) region and is subdivided into the subregions AZFa, AZFb, AZFc (Fig. 6). More recent data confirms that the AZFb and c regions are overlapping. The deletions develop by recombination between two palindromic sequences, which occur in various segments within the AZF region.

In general, deletions of AZFa result in the Sertoli Cell Only Syndrome (SCOS), i. e. azoospermia with complete loss of germ cells in the testes. Thus, TESE (testicular sperm extraction) for assisted reproduction purposes is not possible. Deletions of AZFb cause spermatogenic arrest. AZFc deletions cause a variable phenotype ranging from oligozoospermia with all meioses stages to SCOS. Deletions in the AZFc region as the single causative of infertility allow for treatment with in vitro fertilization via intracytoplasmic sperm injection after TESE. Men with AZF deletions and successful TESE have a normal fertilization and pregnancy rate. The risk for congenital abnormalities in the newborn is not increased. However, in male newborns, fertility problems are to be expected.

The prevalence of Y chromosomal deletions is estimated to be between 1:250 and 1:3000. The prevalence of all AZF deletions in men with non-obstructive azoospermia lies between 8% and 20%, with severe oligozoospermia between 5.5% and 10% and among healthy men at 0.6% to 3.2%. The frequency of deletions for the AZF intervals is 59.6% for AZFc, 15.8% for AZFb, 4.9% for AZFa. The remaining deletions comprise more extensive regions with two or three intervals. In 6% of infertile men with deletions in the Y chromosome, the mutations lie beyond the three intervals.

The precise characterization of the AZF deletion thus has prognostic value and influence on the therapeutic strategy. For counseling, it is important to emphasize that in assisted reproduction, the male offspring inherits the mutation and thus is likely to experience fertility issues as well.

Cystic fibrosis gene mutations

In cystic fibrosis, an autosomal-recessive disorder, patients are either homozygous or compound heterozygous for CFTR (Cystic fibrosis transmembrane conductance regulator) mutations. Certain mutations of the CFTR gene are associated with bilateral congenital aplasia of vas deferens (CAVD) with or without manifestation of CF. Isolated CAVD due to a CFTR gene mutation is classified as CF related disease (CF-RD). CFTR mutations can also result in unilateral aplasia of the vas deferens (AVD), which results in severe oligozoospermia (≤1 million spermatozoa/ml).

Screening data from patients with CAVD without renal anomalies or CF symptoms show that in approximately 10% of cases, one CFTR allele is mutated while the second mutation remains unidentified. Approximately 74% of patients carry two mutated alleles. In those cases, 88% are compound heterozygotes carrying one mild and one severe mutation, while 12% show a mild mutation in both alleles. Considering possible prenatal testing, it is important to also test the partner for mutations in the CFTR gene.

The most frequent CFTR gene mutations which cause isolated CAVD is a delta F508 /R117H combination. In Germany, approximately 21.5% of all tested CAVD patients are carriers of this mutation whereas 8.6% have a F508del/IVS8-5T constellation. The IVS8-5T allele, also known as the T5 allele, is a particularity and a suitable example for the increasing complexity of genetics. The determining nucleotide sequence of the 5T allele is located in intron 8 at the acceptor splice site (Fig. 7), a sequence that serves as an interface to the correct splicing of non-coding RNA sequences (introns).
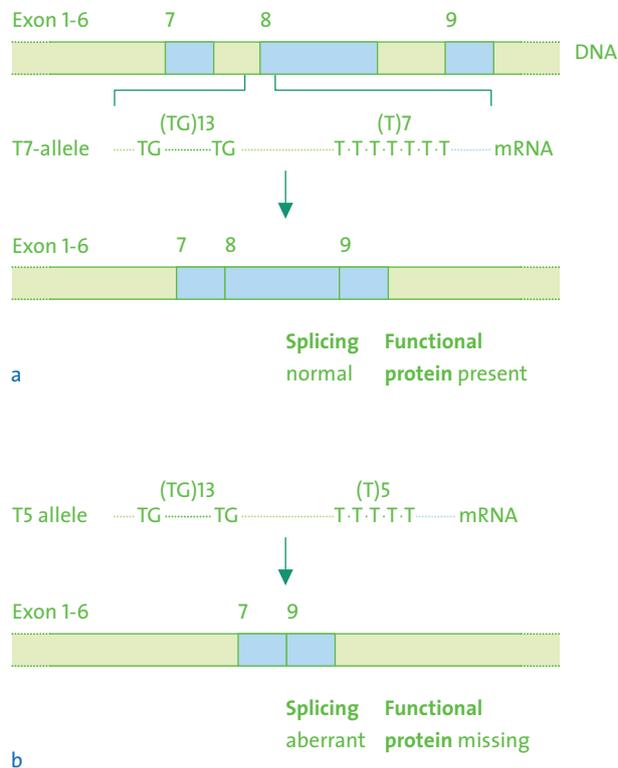

Figure 7: Structure of the CFTR gene and position of the T5, T7 and the (TG)13 variants. The influence of two combinations of the alleles on mRNA splicing is outlined: normal splicing (a), aberrant splicing (b)

Table 2: Influence of different combinations of the T and TG variants on the amount of functional protein and the corresponding clinical manifestations; VDA: aplasia of the vas deferens; CF: cystic fibrosis

TG	T	Amount of functional protein	Clinical symptoms
(TG)11-15	T9	sufficient	none
(TG)11-15	T7	sufficient	none
(TG)11	T5	reduced	VDA
(TG)12	T5	reduced	VDA to mild CF
(TG)13	T5	insufficient	CF

The polythymidine tract can exist in three variants: 5T allele (5 thymidines) 7T allele (7 thymidines) or 9T allele (9 thymidines). The 5T allele results in the lack of exon 9, as the splicing signal located at the end of intron 8 is not recognized, resulting in exon-9 skipping. Transcripts missing exon 9 lead to nonfunctional proteins.

The amount of functional protein is directly proportional to the number of thymidines. In the 7T and 9T tracts, functional protein is sufficiently produced. However, even in carriers with the 5T allele, a pathological reduction in functional CFTR concentration is not obligatory, i.e. an example of incomplete penetrance. The reason is an adjacent TG tandem repeat, which influences disease penetrance. The higher the number of TG repeats, the lower the splicing efficiency (Fig.7). There are (TG)11, (TG)12, (TG)13 and, rarely, (TG)15 repeats. Depending on the combination of T and TG alleles, the clinical phenotype can range from a full-blown CF phenotype [(TG)13T5] to an isolated CAVD [(TG)11T5] or even a normal phenotype (Table 2).

CAVD or unilateral AVD with severe oligozoospermia or azoospermia should always be an indication for molecular genetic testing of the CFTR gene. Other reasons for testing include a lower ejaculate volume (< 2 ml) with a pH < 7.2 and reduced fructose and α-1.4-glucosidase levels. If an IVF with ICSI is intended, it is sensible to test both partners for CFTR gene mutations, considering the possibilities of prenatal testing.

Further mutations and syndromes
The prevalence of syndromic genetic diseases causing infertility is stated to be 1.9% compared to 0.9% in the control group.

The majority of these result in severe congenital abnormalities and mental retardation and patients usually don't approach the doctor for family planning purposes. However, some syndromes are characterized by a spectrum of mild, less pronounced or late-onset symptoms, in which infertility or subfertility are the main focus. These genetic diseases are rather unknown, however, significant in the assisted reproduction context and are best diagnosed in the course of genetic counseling accompanied by further testing.

One example is the androgenital syndrome, which was described above in the context of female fertility issues. Here, only the clinically latent adrenal hyperandrogenism of the male is briefly mentioned. If these patients are not under long-term glucocorticoid treatment, fertility is impaired. Most patients are compound heterozygotes.

Couples with recurrent abortions
The term recurrent or habitual abortion has a different connotation when used by a gynecologist compared to a human geneticist.

According to the WHO, habitual abortions are defined as three consecutive miscarriages. This definition has widely been adopted by gynecologists. In the course of genetic counseling and testing, recurrent abortions are defined as two or more abortions in the familial history, not considering any successful pregnancies. Referring to current literature, this population has a risk of about 3% to carry a balanced chromosomal aberrations in one parent, independent of whether a healthy child was born or not. Thus, prior recommendations that only commence genetic testing after three abortions, have become obsolete. If an abortion is combined with a stillbirth or a child with malformations with or without mental disabilities, the risk for balanced chromosomal aberrations increases to approximately 5.5%. If the familiar anamnestic data provide additional occurrence of abortions or of children with malformations, the risk to possess a chromosomal abnormality increases to 10%.

In the following passages, only the most important genetic reasons for abortions are presented; a short overview of other factors, i.e. anatomical, infectious, endocrinological, immunological and exogenous can be found in Pildner von Steinburg and Schneider (2009).

Chromosome analyses
Usually, chromosomal abnormalities are mainly balanced translocations, which are easily detectable with light microscopy (Fig. 4) and only seldom inversions or insertions. In general, these structural chromosomal abnormalities are without consequences for the carrier himself, as there is no loss of genetic material. Thus, carriers of balanced translocations are not discovered with a physical examination but only with chromosome analyses. Thus, chromosomal karyotyping is indicated in couples with two or more miscarriages.

Patients with balanced chromosomal abnormalities carry a high risk for the development of unbalanced gametes, which lead to imbalanced embryos and fetuses. Common consequences are abortions, but term pregnancies with a child with mental disabilities and/or malformations is possible, especially in cases with a parent showing smaller translocation segments.

When balanced chromosomal anomalies are detected, genetic family counseling has got to be offered and invasive prenatal testing should be discussed. In Germany, only Preimplantation Genetic Diagnosis (PGD) by polar body analysis is legal, thus restricting the analysis to the female chromosomal set.

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Molecular analyses

Hereditary thrombophilic factors are genetic modifications that cause thromboembolic events and predispose to recurrent abortions. There is a clinically proven correlation between the occurrence of abortions and mild hyperhomocysteinemia as well as characteristic mutations of the FV and the FII mutations. Two specific mutations, the FV Leiden mutation (A506G) and the FII (G20210A) mutation, are especially clinical relevant.

Depending on whether the FV Leiden carrier is heterozygous or homozygous, the risk for thrombosis is 4- to 8-fold or 15- to 30-fold increased. The risk for abortions is 2- to 3-fold or 3- to 5-fold increased. The FII mutation increases the risk for thrombosis 2- to 4-fold in heterozygous and 12-fold in homozygous carriers. Here, the risk for abortions is 2-fold increased in heterozygous carriers.

There are controversies regarding the effects of the C667T and the A1298C polymorphisms in the MTHFR gene. In homozygous carriers of the C677T allele, a mild as well as a missing association with abortions were determined. The controversy can be explained by the complex interactions of the thrombophilic factors amongst each other and with other factors, such as environmental factors. Thus, the exact influence of a single factor is difficult to define, as coexistent genetic and non-genetic thrombophilic factors have an additive or even a supra-additive effect.

Compound heterozygosity for the C667T and A1298C polymorphisms can cause hyperhomocysteinemia and be associated with abortions. In summary, in couples with indications for thrombophilia in the familial history or in couples with recurrent abortions and a normal chromosomal set, molecular genetic testing of Factor V and Factor II is indicated. A positive result in the MTHFR analysis of genetic polymorphism should always be associated with testing of the homocystein levels in order to estimate clinical relevance.

Genetic testing of aborted fetal tissue

Abortions are frequent incidences. One in six or one in seven confirmed pregnancies result in abortions. Most of these abortions occur within the first trimester. The reasons for abortions are various and cannot be disclosed in a number of cases. From the mother's side, immunological or fetal factors are relevant. The most frequent reason for abortions are chromosomal anomalies, diagnosed in 50% to 60% of all abortions in the first trimester and in 20% in the second trimester. These chromosomal abnormalities are usually trisomies, generally caused by chromosomal malsegregation.

Some publications request prenatal testing after the confirmation of the trisomy in an abortion, as the risk for recurrence is increased for the following pregnancies. However, this small increase in risk has to be related to the basic risk and may apply to younger women. In contrast, this additional risk has only very limited practical significance in elderly women. This becomes evident in the prognosis for a healthy child in subsequent pregnancies: the confirmation of trisomy in fetal tissue after abortion greatly increases the prospects for a healthy child compared to an abortion, in which the etiology remains unidentified (Table 3).

Table 3: Karyotype of abortion and outcome of the subsequent pregnancies

Karyotype of abortion	n	Successful subsequent pregnancy	Successful subsequent pregnancy (%)	Significance	Authors
Normal	71	17	38.0		
Pathological	60	37	61.7	P = 0.001	*
Normal	39	16	41.0	OR 3.1	
Pathological	18	12	66.7	(95%-CI: 0.85-11.74)	**

*Ogasawara et al. 2000; **Carp et al. 2001

The availability of cytogenetic analysis of the abortion enables more precise genetic counseling and significantly reduces the psychological burden for the patient. The confirmation of a chromosomal malsegregation by chance as the cause of an abortion is usually a great comfort for the parents.

In order to analyze abortion tissue, untreated tissue is necessary for cell culture. The tissue may never be in contact with formalin or disinfectants, as this causes cell destruction and no proliferation could take place.

Depending on the growth potential of the tissue, the results arrive after 4 to 30 days. A structural analysis in a numerically normal chromosomal set is indicated, but limited by the low band resolution in aborted tissue. Major but to that point undetected balanced chromosomal aberrations of the parents, however, may be revealed by the diagnosis of a derivative or recombinant chromosome in abortion tissue.

Results of molecular cytogenetic (Array-CGH) or molecular genetic analysis in abortion tissue have already been published. However, the data do not allow for a clear-cut positioning of its prognostic value, especially concerning the cost-benefit ratio of the (still) relatively expensive diagnostic technology.

Conclusion

Genetic testing in involuntarily childless couples may contribute to the clarification of the cause(s). Early genetic counseling prior to genetic testing is recommended or will be requested by law in certain cases in the near future. On the basis of the results, molecular genetic analyses may be indicated. Examination of fetal tissue after abortion is important in the psychological support of the couples and of particular value in the prognosis for the next pregnancy.

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Prof. Dr. rer. nat. Rolf-Dieter Wegner

Zentrum für Pränataldiagnostik
Kurfürstendamm 199
10719 Berlin

Professor Wegner studied biology at the Freie Universität Berlin, where he later on wrote his Habilitation. He was head of the cytogenetic diagnostic department at the genetic counseling unit in Berlin, part of the Institute for Human Genetics of the Freie Universität Berlin, later known as Klinikum Charlottenburg. Professor Wegner is apl. Prof. (tenured University lecturer) at the Institute for Human Genetics, Charité, Campus Virchow. Since 1999, he has been working in the field of human genetic testing in the practice "Zentrum für Pränataldiagnostik, Kudamm 199".

His professional focus is on prenatal genetic testing, genetic testing in desired pregnancy and genetic testing in pediatrics.

Conflict of interest
The author declares that there is no conflict of interest as defined by the guidelines of the International Committee of Medical Journal Editors (ICMJE; www.icmje.org).

CME

Genetic testing in couples with infertility

Question 1
When should chromosome analysis in couples with miscarriages take place?
a. after the first miscarriage
b. after the second miscarriage
c. after more than two miscarriages
d. Chromosome analysis is not necessary if the first pregnancy was successful and the child healthy.
e. Chromosome analysis is not necessary if in between three miscarriages, the couple gave birth to a healthy child

Question 2
What type of chromosomal abnormality is the most common one in couples with recurrent abortions?
a. Deletion
b. Translocation
c. Duplication
d. Inversion
e. Insertion

Question 3
What kind of diagnostics would you apply when suspecting Klinefelter syndrome?
a. Chromosome analysis
b. FISH analysis
c. DNA analysis
d. Chromosome analysis and FISH analysis
e. Chromosome analysis und DNA analysis

Question 4
You see a 20-year old patient with mild Turner syndrome symptoms and an irregular menstruation cycle. Which chromosomal set best fits the clinical picture?
a. 45,X
b. 47,XXX
c. 47,XXY
d. mos 45,X/46,XY (Turner syndrome mosaicism)
e. mos 45,X/46,XX (Turner syndrome mosaicism)

Question 5
A woman presents with preterm ovarian failure and increased FSH levels. What type of testing should be initiated?
a. CF testing for mutations in the CFTR gene
b. chromosome analysis alone is sufficient
c. testing for mutations in the FMR1 gene
d. chromosome analysis and, in the case of normal results, FMR1 gene testing
e. chromosome analysis and, in suspicious findings, FMR1 gene testing

Question 6
The AZF region on the Y chromosome contains relevant genetic information for
a. Female sex determination,
b. male sex determination,
c. development of the central nervous system,
d. development of functional oocytes,
e. development of functional sperm.

Question 7
The products of the CFTR gene are relevant for the
a. Development of the ovaries,
b. development of secondary sex determinants,
c. development of the vas deferens,
d. development of the oocytes,
e. development of sperm.

Question 8
In a patient with desire for a child and signs for the beginning premature ovarian failure, you confirm a premutation of the FMR1 gene. Which of the following aspects will have to be discussed in genetic counseling?
a. The patient can develop mental retardation.
b. The premutation is associated with the fertility problems.
c. The premutation is not associated with the fertility problems.
d. The premutation is associated with fertility problems. In the case of a pregnancy, the development of a full mutation cannot be excluded. Especially in male fetuses, there is a risk for the development of fragile X syndrome. There is a risk for late-onset progressive neurodegenerative disease.
e. The premutation is associated with the fertility issues. In the case of a pregnancy with a male fetus, the development of a full mutation, i. e. the clinical symptoms of fragile X chromosome, are excluded. There is a risk for late-onset progressive neurodegenerative diseases.

Question 9
Chromosome analysis in aborted tissues
a. has no value,
b. has prognostic value,
c. can reduce the psychological burden of the
   couple,
d. has diagnostic value
e. b, c and d are correct.

Question 10
Which of the following gene combinations can be
associated with abortions due to a genetic
mutation in both genes?
a. CFTR and CYP21
b. CFTR and FMR1
c. F5 (factor V) and CYP21
d. F5 (factor V) and F2 (factor II)
e. F5 (factor V) and FMR1