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Review

Genetic causes of male infertility

Alberto Ferlin, Barbara Arredi, Carlo Foresta *

University of Padova, Department of Histology, Microbiology and Medical Biotechnologies, Centre for Male Gamete Cryopreservation, Via Gabelli 63, 35121 Padova, Italy

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Abstract

Genetic causes account for 10–15% of severe male infertility, including chromosomal aberrations and single gene mutations. Natural selection prevents the transmission of mutations causing infertility, while this protective mechanism may be overcome by assisted reproduction techniques. Consequently the identification of genetic factors has become good practice for appropriate management of the infertile couple. Furthermore, patients affected by some forms of genetic alterations produce a higher frequency of sperm with aneuploidies. Sperm aneuploidies are the direct result of the constitutional genetic abnormality or are caused by meiotic errors induced by the altered testicular environment that these men present. In this review we will report and discuss the genetic causes of male infertility known up to date and we will analyse genetic polymorphisms possibly associated with male infertility.

Keywords: Male infertility; Genetics; Y chromosome; Klinefelter; Androgen receptor; Polymorphism

Contents

* Corresponding author. Tel.: +39 049 8218517; fax: +39 049 8218520.
E-mail address: carlo.foresta@unipd.it (C. Foresta).

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1. Introduction

Infertility affects about 15% of couples trying to conceive in Western countries [1], and genetic causes may be identified in a large proportion of infertile couples. In about 15% of male and 10% of female infertile subject genetic abnormalities could be present, including chromosome aberrations and single gene mutations. Intracytoplasmic sperm injection, the injection of a single sperm into a single egg, is one of the leading methods of treatment for male factor infertility. However, patients affected by some forms of genetic abnormalities (for example chromosomal alterations and Y chromosome microdeletions) produce a higher frequency of sperm with aneuploidies. Sperm aneuploidies are the direct result of the constitutional genetic abnormality or are caused by meiotic errors induced by the altered testicular environment that these men present. The risk of transmission concerns also the use of standard in vitro fertilization (IVF) and intrauterine insemination (IUI), since also normozoospermic infertile men could have some genetic defect (for example 47,XYY males). Natural selection prevents the transmission of mutations causing infertility, while this protective mechanism is overcome by the assisted reproduction techniques. Consequently the risk consists in the increase of the genetic causes of infertility in the future, and thus, the identification of genetic factors has become good practice for appropriate management of the infertile couple.

Recently, we prepared guidelines for the appropriate use of genetic tests in the infertile couple [2]. These guidelines have been prepared not to include all the genetic causes of infertility, but only those clinically relevant, both in terms of prevalence in male and female infertility and risk of transmission to offspring.

In this review we will report and discuss the genetic causes of male infertility know up to date, and finally, we will analyse genetic polymorphisms possibly associated with male infertility (Table 1).

2. Chromosomal abnormalities

The prevalence of chromosome abnormalities is higher in infertile men, this figure being inversely related to the sperm count. Based on the largest published series it could be estimated that the overall incidence of a chromosomal factor in infertile males ranges between 2% and 8%, with a mean value of 5%. This value is increasing to about 15% in azoospermic males, being largely contributed by patients with 47,XXY aneuploidy. Sex chromosomes abnormalities are predominating, but a wide range of structural autosomal anomalies are also found. Preliminary results on pregnancies conceived through ICSI suggest that sex chromosome anomalies may be more frequent than in the naturally occurring pregnancies. In general, children conceived by ICSI have an increased risk of an abnormal karyotype. Although data are still controversial, a figure of about 3% has been suggested, half of them being transmitted by a chromosomally abnormal prospective father.

The most common type of karyotype abnormality detected in infertile subjects is represented by Klinefelter syndrome, and the most frequent non-chromosomal alteration is represented by Y chromosome long arm microdeletions [2]. In a series of 750 consecutive severe oligozoospermic men (sperm count <5 million/ml) collected in our Centre from 1996 [3], 42 subjects (5.6%) had chromosomal aberrations and 45 (6.0%) had Y chromosome microdeletions. The majority of abnormal karyotypes were of the Klinefelter's type (29 cases); the majority of Y chromosome microdeletions were of the AZFc subtype (32 cases).

Table 1
Frequency and associated phenotypes of the most common genetic abnormalities associated with male infertility

Genetic abnormality	Phenotype	Prevalence
Chromosomal aberrations	From azoospermia to normospermia	2–10%
Klinefelter syndrome	Azoospermia–severe oligospermia	5–10% azoospermia; 2–5% severe oligospermia
Other sex chromosome alterations	From azoospermia to normospermia	0.1–0.2%
Robertsonian translocations	Azoospermia–severe oligospermia	0.5–1.0%
Reciprocal translocations	Azoospermia–severe oligospermia	0.5–1.0%
Y chromosome deletions	Azoospermia–severe oligospermia	5–10%
AZFa	Azoospermia-SCOS	0.5–1.0%
AZFb	Azoospermia-spermatogenic arrest	0.5–1.0%
AZFc	Azoospermia-severe oligospermia	3–7%
AZFb-c	SCOS/Spermatogenic arrest	0.5–1.0%
Partial AZFc deletions	From azoospermia to normospermia	3–5%
Gene mutations		
CFTR	Obstructive azoospermia	60–70% (5% in infertile men)
AR	Azoospermia–oligospermia	2–3%
INSL3-LGR8	Cryptorchidism	4–5%

The prevalence shown is the frequency found in the associated phenotype. SCOS: Sertoli cell only syndrome.

Peripheral blood karyotype analysis is therefore strongly recommended during the diagnostic workup of subjects with azoospermia and severe oligozoospermia. Cytogenetic screening is mandatory prior to any assisted reproduction techniques procedure (including intrauterine insemination), also in those cases in which sperm parameters are within the normal ranges or only slightly abnormal. In fact, some karyotype anomalies (for example 47,XYY, some translocations and other structural aberrations) may cause male infertility associated with an apparent normozoospermia.

2.1. Klinefelter syndrome and XXY mosaics

Klinefelter syndrome (KS) is the most frequent sex chromosome aneuploidy in human males, occurring in approximately 0.1–0.2% in newborn males. The prevalence of KS among infertile men is very high, up to 5% in severe oligozoospermia and 10% in azoospermia. KS is a form of primary testicular failure with testicular hypotrophy and elevated gonadotropin plasma levels, and it represents the most common form of male hypogonadism.

It has been always assumed that more than 90% of non-mosaic 47,XXY males are azoospermic. In our series, 72 of 94 (76.6%) non-mosaic KS had complete azoospermia, whereas the remaining had sperm in the ejaculate. Although clear data are not available, it is also possible that a fraction of azoospermic KS men might actually have residual spermatogenesis in some seminiferous tubules [4]. Mosaic 47,XXY/46,XY patients produce spermatozoa in variable numbers. Although the exact percentage of men with sperm in the ejaculate is not known, in our series 20 of 27 (74.4%) were azoospermic.

Before the introduction of ICSI, the fertility outlook for the vast majority of KS patients was hopeless. To date 54 normal children have been born from 122 men with KS by ICSI with testicular (48 children, 118 patients) or ejaculated spermatozoa (6 children from 4 patients) (reviewed in [5]). Although the great majority of children born to fathers with KS are chromosomally normal, the risk of producing offspring with chromosome aneuploidies is significant, particularly the risk of fathering a 47,XXY or 47,XXX child [6,7]. In fact, the incidence of aneuploid spermatozoa is increased in KS. Aneuploid spermatozoa are probably the result of meiosis of few 47,XXY spermatocytes and of meiotic abnormalities occurring in normal 46,XY germ cells present in a compromised testicular environment [5].

2.2. Other sex chromosomes aneuploidies

The karyotype 47,XYY is the second most frequent full aneuploidy of sex chromosomes. Most of the studies preformed by fluorescent in situ hybridisation (FISH) on spermatozoa of XYY males shown a moderate increased frequency of sex chromosome abnormalities. However, no evidence for an increased risk for aneuploidy in the progeny of 47,XYY males have been found.

46,XX chromosomal abnormality is observed mainly in azoospermic males, with frequency of 0.9% [8]. The phenotype is similar to Klinefelter syndrome, but with normal height and unimpaired intelligence. The *SRY* gene is present in most of the cases (SRY^+ XX males); in these cases males are invariably infertile, and azoospermia results from testicular atrophy. The other category are SRY^- XX males, which assumes a mutation in an autosomal or X-linked gene involved in the sex determining cascade which should substitute the SRY, permitting testicular determination in absence of SRY.

The frequency of Y chromosome rearrangements is elevated in infertile men, especially with azoospermia. The association of the infertile phenotype with rearrangements involving the band Yq11.23 (the AZF region) (inversions, deletions of the euchromatic part of the Yq), has been extensively analysed [9]. The loss of partial Yq regions is not detectable by karyotype analysis, and will be discussed later.

2.3. Translocations involving the sex chromosomes

The phenotype or fertility effects of X-autosomal translocations vary depending on the sex carrier, the portions of breakpoints and the pattern of X inactivation. Usually female carriers remain fertile, even if gonadal dysgenesis may occur. In males usually azoospermia is present.

The frequency of Y-autosomal translocations have been reported to be 1 in 2000 [10]. A slight preponderance was observed in the oligozoospermic group (0.2%) and in the ICSI group (0.09%) [8]. Breakpoints in the Y chromosome were prevailing in the q arm at band q11. According to the results reported in [9], in fertile males the breakpoint of the Y chromosome is in Yq12 (the heterochromatic region) and in sterile males the breakpoint is assumed to be in the distal Yq11 euchromatic region at the azoospermic factor (AZF) locus.

2.4. Robertsonian translocations

Robertsonian translocations are the most frequent structural chromosomal abnormalities in humans and can affect fertility, with various degrees of sperm alterations in men. Robertsonian translocations occur when two acrocentric chromosomes (13–15, 21, 22) fuse together. The resulting single abnormal chromosome, generally dicentric, contains most of the long arms of the original two and subsequent loss of their short arms. Balanced translocation carriers have only 45 chromosomes. The incidence of robertsonian translocations is ∼1 in 1000 newborns [11]. The most common combinations are between chromosomes 13 and 14 and between chromosomes 14 and 21. Robertsonian translocation can occur de novo in ∼50% of cases or be transmitted by the mother or the father. Carriers of the robertsonian translocation generally have normal phenotypes. But the translocation can affect fertility and/or pregnancy outcome due to possibly impaired gametogenesis and/or production of gametes with an unbalanced combination of the parental rearrangement. Fertility problems in robertsonian translocation male carriers are due to various degrees of spermatogenic defects directly related to the disturbance of the meiotic process. In populations of infertile males 0.8% were carriers of a robertsonian translocation [12,13], this is up to nine times higher than in the general population.

Studies of the segregation process in robertsonian carriers showed a predominance of alternate segregation resulting in the production of a predominance of normal/balanced spermatozoa, with a percentage of unbalanced spermatozoa varying from 3.4% to 40% (mean 14.57%). Furthermore, recent studies suggest an interchromosomal effect, with sperm aneuploid not only for the chromosomes involved in the translocation, but also for other chromosomes.

2.5. Reciprocal translocations

Reciprocal translocations are found with a frequency of 0.9/1000 newborn. A translocation consists of a mutual exchange of chromosomal segments between two chromosomes. In general, there is no apparent alteration to the carrier's phenotype.

In couples experiencing repeated pregnancy losses, the incidence of chromosomal translocations is higher than the incidence present in newborn series [13]. On the other hand, there is also evidence which indicates that the presence of translocations alters the spermatogenic process. Summarizing the findings from different series of studies on infertile, oligozoospermic and azoospermic males, the incidence of reciprocal translocation carriers is seven times more elevated than in newborn series.

As a general rule reciprocal translocation carriers produce more unbalanced sperm than normal or balanced sperm. The proportion of unbalanced forms depends on the characteristics of the reorganization and it varies widely (from 23% to 81%). Thus it is important to perform a detailed meiotic behaviour analysis for each particular translocation in order to obtain enough information to give adequate genetic counseling.

3. Y chromosome deletions

3.1. Y chromosome microdeletions

From the initial observation in 1976 [14] a number of studies ascertained that microdeletions in the Y chromosome (Yq) represent the most frequent molecular genetic cause of severe infertility, observed with a prevalence of 10–15% in non-obstuctive azoospermia and severe oligozoospermia [15]. Three regions, referred to as "azoospermia factors" (AZFa, b and c from proximal to distal) has been defined as spermatogenesis loci [16]. The genetic pathways and mechanisms of spermatogenic impairment in men with Yq microdeletions are unknown. The function of AZF genes in spermatogenesis is not clear, and also the molecular mechanism altered in cases of AZF deletions are completely unknown. The majority of Y microdeletions produce the simultaneous loss of several genes mapped within AZFb and AZFc loci [17–19]. AZFa deletions are less frequent and involve only two gene, USP9Y and DBY. Most of the AZF microdeletions are generated by intrachromosomal homologous recombination between repeated sequence blocks organised into palindromic structures showing a nearly identical sequence [17]. The complete AZFc deletion, b2/b4 deletion, removes eight gene families including all members of the DAZ gene family, that represent the strongest candidate responsible for the AZFc phenotype [9,15,17,20–23]. Deletions in AZFa region usually lead to Sertoli cell-only syndrome, complete deletions of AZFb or AZFb + c lead to azoospermia associated with Sertoli cell-only syndrome or pre-meiotic spermatogenic arrest [15,23]. The most frequent AZFc deletion leads to azoospermia or severe oligozoospermia, associated with different spermatogenic phenotypes in the testis. In general, about 60% of these men have sperm in the ejaculate or in the testis.

It is still not clear whether the phenotype caused by these rearrangements is caused by the loss of all genes involved in the deletion, or by the disruption of a major gene, whose deletion alone would be able to induce the spermatogenesis failure. Moreover, the presence of variable phenotype, ranging from oligozoospermia to azoospermia, in patients showing apparently identical microdeletions suggests the presence of other modifier genes able to produce different genetic backgrounds, which in turn could balance or enhance the molecular effect produced by the loss of genes mapped within AZF loci.

Most men with Yq microdeletions require ICSI (with ejaculated or testicular spermatozoa) to overcome their infertility. Since all spermatozoa from Y-deleted men harbour the same microdeletions [24], ICSI allows the transmission of such microdeletions. Male offspring of men with Yq microdeletions will therefore also carry the deletion and will have spermatogenic impairment in adulthood. We recently reported the study of 11 oligozoospermic men with AZFc deletions [3] to verify the presence of sperm aneuploidy. Taken together, patients with AZF deletions had a significant reduction in the percentage of normal Y-bearing spermatozoa with respect to normozoospermic control men (33.3 ± 3.2 versus 49.0 ± 1.7, $P < 0.01$), and a concomitant increase in nullisomic sperm (11.9 ± 3.2 versus 1.1 ± 0.2, $P < 0.01$). We also found a significant increase of XY-disomic sperm (4.1 ± 1.2 versus 0.2 ± 0.5, $P < 0.01$). The high frequency of nullisomic sperm even in men with submicroscopic deletions of the Y chromosome, suggests a more general instability of the Y chromosome that could be more pronounced in germ cells than in somatic ones. Therefore, AZF microdeletions can be considered as "pre-mutations" for a subsequent complete loss of the Y chromosome in the AZF deleted patients' sperm, increasing the risk of embryonic X0 cells [25].

Although no genital abnormalities or other somatic defects in the ICSI-AZFc offspring are reported, genetic counselling should take into account the observations of sperm sex chromosome aneuploidies in these men, and the possible increased risk of generating 45,X (using nullisomic sperm) or 47,XXY embryos (using XY-disomic sperm). Pre-implantation diagnosis and long-term follow-up studies of children conceived from men with Y chromosome microdeletions by ICSI are needed to verify these hypothesis.

3.2. Partial AZFc deletions

As mentioned above, AZFc deletions including all members of DAZ gene family represent the most frequent molecular cause of spermatogenic impairment. More recent data suggested that other intrachromosomal recombinations within AZFc could be associated with an increased risk of spermatogenic failure.

Apart from initial studies reporting partial DAZ deletions, performed by FISH and Southern blotting, a number of studies recently reported partial AZFc deletions analysed by AZFc-specific STSs, DAZ-specific SNVs or gene dosage analysis, allowing also to clarify the underlying deletion mechanism. Different partial AZFc deletions have been identified [35–38]: gr/gr, including also the subtype g1/g2, that removes 1.6 Mb, b1/b3 and b2/b3 that remove 1.8 Mb, and others more infrequent. Partial AZFc deletions may represent a risk factor for spermatogenic failure even if definitive data are still missing [19,36–42]. However, further investigations are needed to clarify the impact of such deletions on male infertility and the molecular mechanisms secondary to the deletion that lead to the spermatogenic impairment. Furthermore, the reliable methods for the identification of partial AZFc deletions are not yet well defined.

4. Gene mutations

Several hundreds of genes are necessary for normal sexual development, testis determination, testis descent, and spermatogenesis. However, only few of them have routine clinical importance. These include the CFTR gene, whose mutations cause cystic fibrosis and absence of vas deferens, the androgen receptor gene, whose mutations cause the androgen insensitivity syndrome and spermatogenic damage, and the INSL3-LGR8 genes, whose mutations have been associated with abnormalities in testis descent (cryptorchidism).

4.1. CFTR gene mutations

The CFTR (cystic fibrosis transmembrane conductance regulator) gene is found in region q31.2 on the long (q) arm of human chromosome 7. About 80% of mutations observed in patients with cystic fibrosis (CF) result from deletion of three base pairs causing the loss of the amino acid phenylalanine located at position 508 in the protein (ΔF508). More than other 900 mutations have been described, all presenting geographical specificity.

Infertility caused by obstructive azoospermia has been reported in >95% of men with CF. There is general agreement that 60–70% of patients with congenital bilateral absence of the vas deferens (CBAVD) have mutations in the CFTR gene, with no other clinical symptoms of CF.

In our series of unselected severely oligozoospermic men [3] we found a prevalence of 1.2% (9/750) of CFTR gene mutations. Four of nine patients had unilateral absence or atresia of vas deferens, but in the remaining subjects no abnormality was observed. Similar results have been recently described in the German population [26]: a heterozygous CFTR mutation was observed in 34 of 597 unselected infertile patients (5.7%). Therefore, the frequency of CFTR heterozigosity is about twofold higher than in the general population ($P < 0.0001$).

Subjects with CFTR mutations are good candidate for ICSI, using sperm retrieved from the ejaculate, testis, or epididymis. Spermatogenesis in these patients is normal, and aneuploidy is not increased in the sperm of affected patients. Because of the risk of cystic fibrosis in the offspring of couples in which the female partner is heterozygous for a CFTR mutation, screening for CFTR mutations should be considered before assisted reproduction techniques [3].

4.2. Androgen receptor gene mutation

Androgens and a functional androgen receptor (AR) are essential for development and maintenance of the male phenotype and spermatogenesis. The AR is encoded by a gene located on the X chromosome and consists of eight exons. Mutations in the AR gene cause a variety of defects known collectively as androgen insensitivity syndrome (AIS). Patients with a mild AIS (MAIS) have male infertility as their primary or even sole symptoms.

We found 26 patients carrying AR mutations (20 different mutations) in 1517 oligozoospermic individuals (1.7%), and none in the control group [27]. Importantly, of the 26 men with AR gene mutations, two presented cryptorchidism, one cryptorchidism and hypospadia, one gynecomastia, whereas 22 did not show signs of androgen insensitivity other than spermatogenic impairment. Furthermore, only a minority of infertile males with elevated testosterone and LH (suggestive for androgen insensitivity) had mutations in the AR gene, even thought the higher the ASI (androgen sensitivity index, the product of LH × testosterone), the more likely a mutation in AR.

Therefore, AR gene mutations may play a role as genetic cause of male infertility and are found with a prevalence of about 2% in unselected infertile men. No clear hormonal or clinical data could be used to preselect patients at higher risk of mutations. Thought mild signs of androgen insensitivity may be present in some cases, the largest part of men with AR abnormalities do not differ from the vast majority of infertile males.

4.3. INSL3-LGR8 genes mutations

Insulin-like factor 3 (INSL3), also known as relaxin-like factor (RLF) is a member of the relaxin-like hormone family produced by the Leydig cells. Research on INSL3 in humans has expanded in the last years following the identification, in rodents, of a role for this peptide in the transabdominal phase of testicular descent by acting on gubernaculum [28,29]. A further impulse to this research has been given by the description of the relative receptor, LGR8 (leucine-rich-repeat-containing G protein-coupled receptor 8) [30,31]. The INSL3 gene comprises two exons, with an intron interrupting the C-peptide coding domain, and it is localized in chromosome 19, close to the Janus kinase 3 (JAK3) gene.

A role in human cryptorchidism has been suggested since several mutations in INSL3 and LGR8 leading to amino acid substitution were found. A review of the literature found a prevalence of mutations of 4–5% in men with cryptorchidism or ex-cryptorchidism [32]. Some of these mutations represent common polymorphisms found with similar frequency both in patients and controls, whereas seven of them were detected exclusively in men with history of maldescent (P49S, R73X, P93L, R102C, R102H, N110K in INSL3 and T222P in LGR8). All these mutations were heterozygous, and patients with two mutant alleles or patients compound heterozygotes

for mutations in INSL3 and LGR8 have never been found, in contrast with that observed for rodents, where homozygotes knockout mice are cryptorchid, but heterozygotes are normal.

Moreover, the identification of high levels of circulating INSL3 in adult males and the expression of LGR8 in many tissues arise new question on the endocrinological role of this hormone [33,34]. Apart from the role in testicular descent and cryptorchidism, INSL3 has therefore possible important yet unidentified endocrine and paracrine actions in adults, and deficiency of this hormone may represent an important sign of functional hypogonadism [32].

5. Gene polymorphisms and male infertility

The analysis of polymorphisms in genes involved in spermatogenesis represents one of the most exciting area of research in genetics of male infertility. Polymorphisms or genetic variants in these genes are considered potential risk factors which may contribute to the severity of spermatogenic failure. Several polymorphic variants have been described in association with male infertility. However, these association studies often do not report unique results. This is mainly due to different important aspects: the size and the composition of the study population, the type of polymorphism analysed and the techniques used, the multifactorial condition and heterogeneity of male infertility phenotype, the interindividual variability in the phenotypic effect of causes acting at the testicular level, and the ethnic and geographical differences that contribute to genetic variations.

The phenotypic effects of gene polymorphisms are modulated by other genetic factors or genetic background and environmental factors, providing an important example of the gene-environment interaction in phenotype development. Therefore, it is likely that polymorphisms only in association with a specific genetic background and/or with environmental factors can lead to spermatogenetic impairment or testicular dysfunction.

Polymorphisms in different genes have been studied for possible association with male infertility, but many of them have not been replicated and therefore definitive data are not available. Therefore, only for some polymorphisms sufficient, although not conclusive, data have been produced.

5.1. Androgen receptor (AR) exon 1 polymorphisms

The AR exhibits two polymorphic sites in exon 1, characterized by different numbers of CAG and GGC repeats resulting in variable lengths of polyglutamine and polyglycine stretches in the N-terminus of the AR protein, that seem to modulate AR function. The number of CAG and GGC repeats ranges from about 10 to 35 (with a mean of 21–23) and 4 to 24 (with a mean of 16–17), respectively, in normal men. A number of recent studies have examined the possible link between the length of the CAG repeat and male infertility. The basis for these investigations has been the finding that longer CAG repeat lengths result in reduced AR transcriptional activity both in vivo and in vitro. This observation has led to the hypothesis that longer polyglutamine tracts might possibly be considered a risk factor for male infertility. This is consistent with the finding that polymorphisms in CAG tract length correlate with sperm concentration in normal men [43]. However, previous studies examining CAG repeat number in infertile men have reported conflicting results, with some showing no expansion, and others reporting increased length (but still within the normal range) with respect to fertile control men (reviewed in 44). In particular, studies involving Singaporean, Australian, North American and Japanese subjects found an association between CAG length and male infertility, whereas this was not evident in studies from Europe. These discordant results may reflect the patient ethnicity selection, as the distribution of the number of CAG repeats is lowest in African-Americans, intermediate in whites, and highest in Asians. Furthermore, additional bias may be related to sample size restrictions, choice of the control group and inclusion patient criteria. Only two studies reported the distribution of GGC lengths among the infertile men and found no difference from that in the general population [45,46]. Recently, we have examined the effects of variation in length of both the CAG and the GGC repeat showing that some haplotypes might modulate AR function and might increase an individual susceptibility to infertility [44]. A similar study concluded that the combination of fewer than 21 CAG and 23 GGN repeats conferred a lower risk of infertility to carriers as opposed to those with more than 21 CAG and 24 GGN repeats [47]. Therefore, definitive data on the role of AR exon 1 polymorphisms in male infertility are not yet available, and additional studies in well-defined populations of infertile men of different ethnic origins should be performed.

5.2. Y chromosome haplogroups

Slowly mutating binary markers on the male specific region of the Y chromosome define Y chromosome haplogroups, which are groups of Y chromosomes sharing the same allelic pattern for the same markers, whose phylogenetic relations has been described in a detailed Y-haplogroups phylogenetic tree. A few groups have studied the possible association of Y-chromosome haplogroups with Yq microdeletions or with particular phenotypes of infertility. Absence of significant association between Y haplogroups and Y microdeletions was found in an European sample [48] and in a northwestern European sample [49]. However, in this latter study an association was found in the Danish population. The gr/gr deletions were observed on several haplogroups, among which the D2b haplogroup contained only deleted chromosomes [36]. This same branch of the Y-phylogeny was suggested to be associated with infertility in Japanese men [50], even if this result has not been confirmed [51]. Instead, the b2/b3 deletion and the more precisely defined DAZ3/DAZ4 deletions seems to be completely associated with the haplogroup N, an ancient lineage widespread in northern Europe and in Asia, as also described by our recent work [52]. Two works highlighted a possible association between haplogroups and infertility: the D2b haplogroup in the Japanese and the haplogroup hg 26+ (or the K^{*}(xP) according to the YCC

nomenclature) in a Danish sample [53]. Another study [54] on Italian populations highlighted a significant difference between the control and infertile samples, even if the variability in frequency due to differences among subpopulations was not taken into account.

Therefore, no conclusive results have yet been reached about the role of Y-haplogroups in infertility or in association with Y-microdeletions. Some studies lacked information on the precise infertile phenotype or on the molecular bases of the microdeletions, or a low resolution in the Y-chromosome phylogeny was used.

5.3. MTHFR

The enzyme 5-methylenetetrahydrofolate reductase (MTHFR) is involved in the conversion of homocysteine to methionine. A point mutation in its coding region (C677T) decreases the activity of the enzyme by about 30% in heterozygotes (CT) and 80% in homozygotes (TT). The frequency of the T allele varies greatly among populations, ranging from 30% to 40% in Europe and Americas to 5–10% in Africans and Sri Lankans [55,56]. The most common phenotype of this mutation is the accumulation of homocysteine leading to homocystinuria/homocysteinemia. The phenotypic effect of the mutation is modulated by exogenous factors such as folate supplemented food. Possible negative effects of the MTHFR (C677T) mutation on male fertility might be due to an alteration in the expression of spermatogenesis genes induced by undermethylation. Alternatively, spermatozoa might be damaged by a higher production of toxic reactive oxygen metabolites causing DNA damage. Up to date six studies have reported the possible association between MTHFR C677T polymorphism and male infertility, but conclusive data cannot be drawn, mainly because of non-homogenous population selection and ethnic differences. In fact, studies from Germany [57], India [58] and Korea [59] showed an association between both homozygous [TT] and heterozygous [CT] MTHFR polymorphism and azoo-oligozoospermia, whereas studies from the Netherlands [60] did not find such association and studies from Italy [61,62] obtained conflicting results.

5.4. DAZL

DAZL is an autosomal homolog of the Y-linked DAZ gene, and it is expressed in the germ cells where it encodes for a RNA-binding protein. No mutations in DAZL gene have been reported so far, except two single nucleotide polymorphisms (SNPs) in exon 2 (A260G) and 3 (A386G). Initial report from Taiwanese men [63] showed that the SNP at position 260 was similarly distributed among infertile and control men, whereas the SNP at position 386, that is located within the highly conserved RNA-recognition motif and that causes a Thr → Ala change at amino acid 54 (T54A), was significantly associated with male infertility. However, subsequent studies from Italy [64,65], Germany [66] and Japan [67] did not confirmed these results. Furthermore, the T54A allele seems not to worsen the phenotype of AZFc-deleted men.

5.5. POLG

DNA polymerase γ is responsible for replication and repair of the mitochondrial genome. Human DNA polymerase γ is composed of a catalytic subunit and an accessory subunit. Mutations in the gene for the catalytic subunit (POLG) have been shown to be a frequent cause of mitochondrial disorders. The human POLG gene contains a 10-unit CAG trinucleotide repeat encoding a poly-glutamine stretch near the N-terminus of the mature protein. Initial studies suggested that alteration of the CAG repeat could be associated with loss of sperm quality and to contribute to 5–10% of the male infertility cases in the European population [68]. However, more recent studies from Italy and France reported that alterations in POLG's CAG trinucleotide repeat was found at the same frequency in both normal and infertile men [69–71]. Therefore, it is not yet clarified whether the POLG CAG-repeat polymorphisms may contribute to male infertility or spermatogenic failure.

5.6. FSHR

The interaction between FSH and the FSH receptor (FSHR) is essential for normal oogenesis and spermatogenesis. Recently, single-nucleotide polymorphisms (SNPs) have been assigned to the FSHR gene. These give rise to different FSHR haplotypes that modify the action of FSH. In exon 10 of the FSHR gene two SNPs are found corresponding to amino acids positions 307 and 680 of the mature protein. These two polymorphisms result in two major, almost equally common allelic variants in the Caucasian population: Thr^{307}-Asn^{680} and Ala^{307}-Ser^{680}. The FSHR polymorphism at position 680 influences serum FSH levels in women and the sensitivity of the FSHR to FSH in vivo. In fact, FSH sensitivities during the menstrual cycle and different cycle lengths are observed, depending on the FSHR haplotype. Furthermore, a different need for FSH is seen in women during controlled ovarian hyperstimulation for in vitro fertilization techniques [72]. Another SNP is located at position-29 of the FSHR gene promoter (resulting in G → A exchange), whose impact, alone or in combination with exon 10 SNPs, is less clear. In men, the impact of the FSHR SNPs is unclear. In particular, a preliminary study [73] showed no differences in the distribution of FSHR polymorphisms between normal and infertile men, whereas a more recent study [74] reported a different allelic frequency in azoospermic with respect to normozoospermic men. It is possible therefore that FSHR haplotypes might represent one of the gene polymorphisms that, alone or in combination, might influence spermatogenesis. The significance of this association needs now to be verified by further studies in other populations, possibly of different ethnic origin.

5.7. Estrogen receptor (ER)α

The physiological response to estrogens are mediated by at least two functional isoforms of estrogen receptor (ER), namely ERα and ERβ, encoded by two different genes. Genetic screening of the ERα gene locus has revealed the existence of several polymorphic sites. The most widely studied are the PvuII and

XbaI restriction fragment length polymorphisms in intron 1, the (TA)n variable number of tandem repeats within the promoter region of the gene and the C → G polymorphism at codon 325 in exon 4. Recent studies suggested an association between ERα polymorphisms and male infertility, although no definitive data can be extrapolated. In Greek population an association has been found with the intronic SNP XbaI and not with PvuII [75], whereas in Spanish men an association was found only for the PvuII polymorphism [76]. The only study analysing exon 4 SNP reported a significantly different allelic distribution between azoospermic and control men in Japanese men [77]. Finally, the [TA]n polymorphism in Italian population, although not differently distributed between infertile and control men, showed an effect on sperm count [78]. Therefore, it is likely that different haplotypes of ERα may contribute to the male infertile phenotype, although additional studied are warranted.

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