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02 May 2026

Symposium: Genetic and epigenetic aspects of assisted reproduction

Gene polymorphisms/mutations relevant to abnormal spermatogenesis

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Abstract

Despite the identification of an increasing number of candidate genes involved in spermatogenesis, the armamentarium of diagnostic genetic tests in male infertility remains extremely limited. A number of new causative mutations have been reported for hypogonadotrophic hypogonadism but still the genetic diagnosis in this pathological condition is made only in about 20% of cases. The sole molecular genetic test that is routinely proposed in severe spermatogenic disturbances is screening for Yq microdeletion. The search for causative mutations in the Y chromosome, and in autosomal and X-linked genes, has mostly been unsuccessful. The paucity of gene mutations raises questions about the appropriateness of the currently used screening approaches. Among the proposed genetic risk factors, gr/gr deletion of the Y chromosome seems to be the most promising polymorphism. Other polymorphisms are awaiting further confirmation, whereas for some (*POLG*, *DAZL*, *USP26*, *FSHR*) a lack of association with abnormal spermatogenesis has now been ascertained. It is likely that some polymorphisms lead to testicular dysfunction only when in association with a specific genetic background or with environmental factors. Future large-scale studies with stringent study design may provide a more efficient way to identify clinically relevant genetic factors of male infertility.

Keywords: *gene mutations, genetic risk factors, gr/gr deletions, male infertility, polymorphisms, spermatogenesis*

Introduction

Spermatogenesis can be altered by different factors acting either at the pretesticular or directly at the testicular level. Despite a significant improvement in the diagnostic work-up of infertile men, in about 50% of cases the cause of abnormal

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spermatogenesis remains unknown and is called idiopathic infertility (Forti and Krausz, 1998). Considering the high number of predicted genes involved in male gametogenesis, it is highly likely that mutations or polymorphisms in candidate genes involved in spermatogenesis are responsible for the majority of idiopathic forms of spermatogenic disturbances.

To date, candidate spermatogenesis genes have been identified mainly through model organisms, expression studies, linkage analysis and cytogenetic findings (for review, see Matzuk and Lamb, 2002). Recently, the approaches of transcriptomics and proteomics have been applied in this field and open a new perspective in understanding male infertility (Ostermeier *et al*., 2002; Martinez-Heredia *et al*., 2006; Platts *et al*., 2007).

This review focuses on data available in the literature on gene mutations/polymorphisms with ascertained or potential clinical relevance in human spermatogenesis.

Genes involved in the endocrine regulation of spermatogenesis

Spermatogenesis and steroidogenesis in the testis is regulated through the hypothalamic–pituitary–testicular axis and involves a number of hormones such as gonadotrophin-releasing hormone (GnRH), FSH, LH, testosterone, oestrogens and inhibin B.

Congenital gonadotrophin deficiency and spermatogenic failure

Mutations in genes responsible for the migration of GnRH neurons, such as anosmin-1 (*KAL1*), fibroblast growth factor receptor 1 (*FGFR1*), G-protein-coupled prokineticin receptor-2 *(PROKR2*), prokineticin-2 (*PROK2*) or mutations in the gonadotrophin-releasing hormone receptor (*GnRH-R*) gene or G-protein-coupled receptor 54 (*GPR54*) cause congenital hypogonadotrophic hypogonadism (for review, see Trarbach *et al*., 2007). Depending on the type of gene involved, these genetic anomalies can be associated with anosmia (Kallmann syndrome) or normosmia. Although sporadic cases are more frequent, families with Kallmann syndrome have been reported with X-linked, autosomal dominant or recessive modes of inheritance. The penetrance of the same mutation may vary in different family members, leading to a wide phenotype spectrum including the presence/absence of non-reproductive and non-olfactory disorders. Gonadotrophin deficiency can also be due to mutations of the beta subunits of LH and FSH. To date, only three distinct FSH and two LH beta subunit gene mutations have been described (Huhtaniemi, 2006). Given that the screening for mutations in all the above-described genes provides the identification of mutations only in about 20% of cases, additional genetic causes are expected to be discovered in the future. The diagnosis of congenital hypogonadotrophic hypogonadism is normally made before adulthood since it is associated with delayed puberty. In adulthood, spermatogenesis can be relatively easily induced by hormonal treatment; congenital hypogonadotrophic hypogonadism therefore represents a peculiar group of treatable forms of spermatogenic disturbance of genetic origin.

Steroid receptor gene mutations

The crucial role of androgens and oestrogens in the endocrine regulation of spermatogenesis is well known; therefore, genes of their receptors are a logical target for mutational analysis in infertile males.

The androgen receptor (AR) is a ligand-activated transcription factor that is encoded by the *AR* gene located on the long arm of the X chromosome (Xq11–q12). A large number of mutations in the *AR* gene have been identified worldwide and are available at the Androgen Receptor Gene Mutations Database (Gottlieb *et al*., 2004). Functional assays have demonstrated that a significant number of these mutations result in lower ligand binding or transactivation potential of the mutant receptor molecule. Mutations in the *AR* gene result in mild-to-complete androgen insensitivity. The phenotypic features of complete androgen insensitivity syndrome are female external genitalia and absence of pubic hair. In partial androgen insensitivity syndrome, several different phenotypes are evident, ranging from predominantly female phenotype (female external genitalia, pubic hair with or without clitoromegaly, and partially to completely fused labia) through ambiguous genitalia to predominantly male phenotype with micropenis, perineal hypospadias and cryptorchidism (Quigley *et al*., 1995). The later phenotype is also termed as Reifenstein syndrome (Quigley *et al*., 1995). Patients with mild androgen insensitivity syndrome have male infertility as their primary or even sole symptom.

Only a few mutations have been reported in male infertility (Gottlieb *et al*., 2004; Ferlin *et al*., 2006) and most of them have resulted in the reduction of transactivation potential of the mutant protein. In a recent screening of 1517 azoo-oligozoospermic individuals, 26 patients carrying *AR* mutations (20 different mutations) (1.7%) were found, and none in the control group (Ferlin *et al*., 2006; Rajender *et al*., 2007). There has been no correlation between the type of mutation and the subtype of infertility (azoospermia, oligozoospermia or oligoteratozoospermia). Moreover, the previously proposed high androgen sensitivity index seems not to be able to predict an accurate selection for *AR* mutation screening. Apart from the difficulty in preselecting patients for the analysis, the need for routine testing in infertile subjects is further questioned by other reports in which none or very few mutations were detected in a large series of idiopathic infertile men (Singh *et al*., 2006; for review see Rajender *et al*., 2007).

The analysis of polymorphic regions in exon 1 of *AR* has been the subject of a large number of publications (Tut *et al*., 1997; Dowsing *et al*., 1999; von Eckardstein *et al*., 2001; Rajpert-de Meyts *et al*., 2002; Foresta *et al*., 2005). The polymorphic $(CAG)_n$ codes for a polyglutamine whereas the $(GGC)_n$ repeat codes for a polyglycine stretch. It has been demonstrated for the $(CAG)_n$ repeats in in-vitro experiments that the length of the polyglutamine tract, while remaining within the polymorphic range, is inversely correlated with the transactivation activity of the receptor (Tut *et al*., 1997). Concerning the $(GGC)_n$ repeat, a recent report showed that *AR* with GGN repeat lengths other than the most common one of 23 have lower transactivating capacity (Lundin *et al*., 2007).

Despite promising in-vitro data, the initial observations of a significant association between relatively long CAG repeats and

impaired sperm production (Dowsing *et al*., 1999) have not been confirmed by subsequent studies (for review see Asatiani *et al*., 2003). Similarly, it is still under question whether the $(GGC)_n$ repeat has a pathogenic role in abnormal spermatogenesis (see Krausz and Giachini, 2007).

Discrepancies in the literature maybe the consequence of: (i) ethnic differences (the association seems to be more consistent in Asiatic populations); (ii) the heterogeneity of the control (unselected men, proven fertile men or normospermic men) and of the infertile (different inclusion criteria) groups; (iii) inadequate sample size (especially in the first positive studies).

In conclusion, mutation screening of neither the *AR* nor the CAG repeats can be proposed as a routine diagnostic test in patients with abnormal spermatogenesis. Mutations of *AR* are rare and many of the mutations described in infertile men still need to be characterized for their functional consequences. Concerning CAG repeat length, this polymorphism is an unlikely risk factor for male infertility if we consider large studies only. However, the role of CAG repeat length in modulating androgen action is more evident in patients affected by hypoandrogenism (for example subjects affected by Klinefelter syndrome) and its analysis could be useful for the definition of thresholds at which testosterone treatment should be initiated, and to personalize the dosage of substitutive testosterone therapy (Zitzmann *et al*., 2004).

Oestrogen receptor

Although human and animal models have shown an association between oestrogen insufficiency and abnormal spermatogenesis, little attention has been paid to the role of oestrogen receptor (*ESR*) gene mutations or polymorphisms in male infertility.

The physiological responses to oestrogens are known to be mediated by at least two functional isoforms of *ESR*, namely *ESR1* and *ESR2*, encoded by two different genes in different chromosomes (6q25 and 14q23–24, respectively). Both receptors share the common structure of the steroid/thyroid hormone nuclear receptor, differing in the C-terminal ligand-binding domain and in the N-terminal transactivation domain (O'Donnell *et al*., 2001). Besides these two isoforms a third membrane receptor has been reported in different cellular models including human spermatozoa (Luconi *et al*., 2002). This membrane receptor is probably involved in the stimulatory effect on sperm capacitation, acrosome reaction, and fertilizing ability of 17β-oestradiol and environmental oestrogens observed in mouse spermatozoa (Adeoya-Osiguwa *et al*., 2004).

To date only one patient with an inactivating mutation in the *ESR1* gene (Smith *et al*., 1994), and four patients with aromatase (CYP19) deficiency have been reported (Morishima *et al*., 1995; Carani *et al*., 1997; Deladoey *et al*., 1999; Maffei *et al*., 2004). The phenotypes of these patients are rather heterogeneous, ranging from normal sperm concentration but reduced sperm viability in cases of the *ESR1* mutation, to oligozoospermia and bilateral cryptorchidism in cases of aromatase deficiency (Carani *et al*., 1997; Maffei *et al*., 2004).

The human *ESR1* gene encodes a protein of 595 amino acids with a molecular mass of about 66 kDa. Genetic screening of the *ESR1* gene locus has revealed the existence of several polymorphic sites. The most widely studied are the *Pvu*II (T397C) and *Xba*I restriction fragment length polymorphisms in intron I and the $(TA)_n$ variable number of tandem repeats within the promoter region of the gene (Gennari *et al*., 2005). Recently a possible relationship between *ESR1* polymorphisms and male infertility has been reported in Greek and Japanese populations (Kukuvitis *et al*., 2002; Suzuki *et al*., 2002). However, due to the relatively small study populations in both studies, the interpretation of these data remains difficult.

The most promising polymorphism is the $(TA)_n$ variable number of tandem repeats within the promoter region. The distribution of the TA genotype is not different between controls and patients, therefore this polymorphism cannot be considered a risk factor for male infertility. However, it has a significant effect on sperm output in both normospermic and infertile men (Guarducci *et al*., 2006). The number of TA repeats showed a significant inverse correlation with sperm count, and consequently men with a higher TA repeat number on both alleles have significantly lower sperm production. A plausible explanation would be that not only a deficit of oestrogens, but also an exaggerated oestrogen action related to this genetic variant (eventually combined with environmental factors), can be deleterious. Since the individual sensitivity to xenoestrogens may depend on the type of *ESR1*, the screening for this polymorphism may be useful in subjects with high exposure to xenoestrogens. Another interesting *ESR1*-gene-related polymorphism is the specific haplotype AGATA, resulting from the allelic combination of five single nucleotide polymorphisms (SNP) situated in a 50 Kb haplotype block of *ESR1*. The AGATA haplotype in the Japanese population has been reported as risk factor for cryptorchidism (Yoshida *et al*., 2005), whereas in the Italian population the tag SNP of the AGATA haplotype (SNP12) has a significant protective effect (Galan *et al*., 2007). The discrepancy between the Italian and the Japanese study may be related to genuine ethnic differences and/or different environmental conditions. A role for *ESR2* gene SNP in male infertility has also been proposed recently (Aschim *et al*., 2005; Galan *et al*., 2005). Given that for each type of variant and haplotype there are only sporadic studies, it is important to confirm their clinical significance in other independent study populations. It would be especially interesting to verify their effect on spermatogenic potential in relation to different grades of exposure to xenoestrogens.

Genes involved in common cell functions

Studies on genes involved in common cell functions that are relevant also for normal spermatogenesis are relatively few and are mainly single studies, with the exception of the mitochondrial DNA polymerase γ (*POLG*) and the methylenetetrahydrofolate reductase (*MTHFR*) genes. To date, only polymorphisms with little or no clinical relevance have been described. The *POLG* gene CAG polymorphism (with a major allele at 10 repeats) has been the subject of an extensive debate in the last few years. POLG is the sole polymerase for mitochondrial DNA (mtDNA) and impaired activity of this protein leads to mitochondrial dysfunction through accumulation of mtDNA mutations. Lack of functional POLG may therefore lead to several cellular dysfunctions including altered cell proliferation. Rovio *et al*. (2001) proposed an association between the absence of the common 10 CAG allele and male infertility in a relatively

small group of infertile and fertile men. Subsequent studies in populations from Europe (Krausz *et al*., 2004; Aknin-Seifer *et al*., 2005; Brusco *et al*., 2006) and New Zealand (Harris *et al*., 2006) failed to confirm this first observation. Similarly, the proposed association with unexplained infertility (Jensen *et al*., 2004) was also rejected after having ruled out interpretation bias (Krausz *et al*., 2004). It is therefore clear that the *POLG* CAG polymorphism has no clinical significance either for idiopathic or for 'unexplained' male infertility.

MTHFR is one of the key enzymes in folate metabolism and reduces 5,10-methylenetetrahydrofolate to its biologically active form 5-methyltetrahydrofolate. The C677T polymorphism of the *MTHFR* gene has been extensively analysed in infertile patients from different populations (Bezold *et al*., 2001; Ebisch *et al*., 2003; Stuppia *et al*., 2003; Singh *et al*., 2005; Park *et al*., 2005). The C677T mutation decreases MTHFR activity and it leads to a decrease in folate levels and an increase in homocysteine levels in the blood. Altered folic acid metabolism influences DNA methylation, and subjects with homozygous mutation 677TT have a lower level of genomic DNA methylation than controls (Stern *et al*., 2000). Since aberrant DNA and protein methylation is likely to affect spermatogenesis, the C677T represent a logical target for mutation screening in patients with impaired spermatogenesis; however, results are discordant. Studies from Indian, African and south-east Asian populations agree that *MTHFR* gene polymorphism is significantly associated with abnormal spermatogenesis, whereas European studies have obtained conflicting results. It is also not clear if only homozygous (TT) or also heterozygous (CT) *MTHFR* polymorphism are of clinical relevance. The most likely explanation is that the polymorphism is relevant only in specific environmental conditions, such as low dietary intake of folates. The pharmacogenomic correlate of the above-mentioned association studies is that idiopathic infertile carriers of the 677TT genotype may potentially benefit from folic acid supplementation.

Genes involved in specific spermatogenic functions

Autosomal and X-linked genes

A number of spermatogenesis autosomal and X-linked candidate genes have been identified (mainly based on animal models) and they represent the most obvious targets for mutation analysis in men with spermatogenic failure. However, the search for candidate gene mutations has not led to the identification of confirmed novel causative mutations for impaired sperm production. The few available examples of 'causative mutations' are reported in **Table 1**, and they are mainly related to the endocrine control of spermatogenesis. In contrast, the number of genetic variants proposed as risk factors for male infertility are constantly increasing. However, in many cases only sporadic data are available and when more studies are published on the same polymorphism the results are often contradictory. Inadequate sample size, pathogenetic heterogeneity of infertility, inappropriate control subjects and ethnic/geographic differences (probably also related to environmental factors) may be responsible for discrepancies among case–control studies. **Table 1** contains a comprehensive list of candidate genes that were screened for mutations in

Table 1. Summary of mutations with cause–effect relationship (causative) and polymorphisms proposed as risk factors for abnormal spermatogenesis.

Mutation	*Gene* *Endocrine regulation of spermatogenesis*	*Specific spermatogenic function*	*Common cell function*
Causative	*AR*, *KAL-1*, *FGFR1*, *PROK2*, *PROK2R*, *GnRH*, *GnRHR*, *GPR54*, *FSH, LH*, *ESR1*, *CYP19*	*USP9Y*, *SYCP3*	
Polymorphisms (single study)	*CYP19A1*, *NRIP1*	*GRTH*, *CREM-ACT*, *KIT-KITLG*, *HANP1/H1T2*, *PUMILIO2*, *BOULE*	*GSTM1*, *PHGPx*, *BRCA2*, *MS*, *MTRR*, *APOB*, *SDHA*
Polymorphisms (more than one study)	***AR***, ***FSHR***, *ESR1*, *ESR2*	***DAZL***, *PRM1–PRM2*, *TNP1–TNP2*, ***USP26***	***POLG***, *MTHFR*

For gene polymorphisms indicated in bold, a role as 'risk factor' has not been confirmed in Caucasian populations. Genes: *ACT* = activator of *CREM* in the testis; *APOB* = apolipoprotein B; *AR* = androgen receptor; *BRCA* = breast cancer; *CREM* = cAMP-responsive element modulator; *CYP19A1* = CYP19 aromatase; *DAZL* = deleted azoospermia-like; *ESR* = oestrogen receptor; *FGFR1* = fibroblast growth factor receptor 1; *FSHR* = FSH receptor; *GnRH* = gonadotrophin-releasing hormone; *GnRHR* = gonadotrophin-releasing hormone receptor; *GPR54* = G-protein-coupled receptor 54; *GRTH* = gonadotrophin-regulated testicular helicase; *GSTM1* = glutathione *S*-transferase M1; *HANP1*/*H1T2* = histone H1-like, nuclear protein 1; *KAL1* = Kallmann syndrome 1; *KIT* = v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue; *KITLG* = KIT ligand; *MS* = methionine synthase; *MTHFR* = 5,10-methylenetetrahydrofolate reductase; *GSTM1* = glutathione *S*-transferase M1; *PHGPx* = phospholipid hydroperoxide glutathione peroxidase; *MTRR* = MS reductase; *NRIP1* = nuclear receptor interacting protein 1; *POLG* = mitochondrial DNA γ-polymerase; *PRM* = protamine; *TNP* = transition nuclear protein; *PROK2* = prokineticin-2; *PROKR2* = G-protein-coupled prokineticin receptor-2; *SDHA* = succinate dehydrogenase, subunit A; *SYCP3* = synaptonemal complex protein 3; *USP26* = ubiquitin-specific peptidase 26; *USP9Y* = ubiquitin-specific protease 9.

infertile men. The few mutations with a cause–effect relationship with abnormal spermatogenesis are already of clinical use in selected groups of patients (congenital hypogonadotrophic hypogonadism, androgen resistance), whereas the clinical significance of polymorphisms (risk factors for abnormal spermatogenesis) and some of the causative mutations are still under debate. A few genes among the most promising ones have been selected for further discussion.

Based on data from model organisms and expression studies, genes expressing the highly conserved family of proteins DAZ, DAZL and BOULE have been proposed as major spermatogenesis candidate genes in humans. The *DAZL* gene is an autosomal homologue of the Y-chromosomal *DAZ* (deleted in azoospermia) gene cluster and maps to chromosome 3p24 (Yen *et al*., 1996). All three family members encode RNA-binding proteins with important roles in spermatogenesis (Yen, 2004). Recently, new insights into the role of DAZL in synapsis formation in meiosis have been obtained (Reynolds *et al*., 2007) based on the observation that Sycp3 is a target of DAZL-mediated translation. Despite extensive mutation screening in the *DAZL* gene, to date only one homozygous mutation in exon 2, in a position close to the RNA-binding domain of the protein, has been found in an azoospermic man (Tung *et al*., 2006b). On the other hand, a high number of SNP have been identified ($n = 85$) and are mainly located in introns or in the 5 and 3 untranslated regions. The only exonic SNP reported as a susceptibility factor to oligo/azoospermia in the Chinese population is located in exon 3 (T54A) (Teng *et al*., 2002). Surprisingly, this variant was not even found in Caucasians (Bartoloni *et al*., 2004; Becherini *et al*., 2004; Tschanter *et al*., 2004). Specific haplotypes, based on the combination of SNP in noncoding regions, have been reported to be associated with sperm count (Tung *et al*., 2006a) or with spermatogenic failure (Teng *et al*., 2006). However, given the discrepancy between these two studies, it remains to be established if such an association does exist and if it is also present in Caucasians. No clinically relevant mutations for the *BOULE* (*BOLL*) gene have been reported (Lepretre *et al*., 2004; Westerveld *et al*., 2005a). Recently, another evolutionary conserved protein, pumilio (PUM2), has been screened for mutations in patients with reduced sperm count (Kusz *et al*., 2007). Despite a clear role of pumilio proteins in germ cell development and the specific expression pattern of *PUM2* in the testis and ovary, the screening did not reveal any causative mutations.

The *SYCP3* gene, located on chromosome 12, encodes a DNA-binding protein as a component of the synaptonemal complex that regulates the synapsis between homologous chromosomes during meiosis of germ cells (Yuan *et al*., 2000). According to the predicted phenotype based on the function of this protein, a null mutation of *Sycp3* in mice causes azoospermia with meiotic arrest. A mutation analysis in highly selected patients affected by maturation arrest identified a 1-bp deletion (643delA) that results in a premature stop codon and truncation of the C-terminal, coiled-coil-forming region of the SYCP3 protein. The same mutation was found in two of 19 patients (Miyamoto *et al*., 2003). Unfortunately, no causative mutations were observed in subsequent European studies in a total of 70 selected patients (Martinez *et al*., 2007) with a similar testicular phenotype to that reported in previously by Stouffs *et al*. (2005a). These later findings exclude a relevant role for *SYCP3* mutations in the aetiopathogenesis of spermatogenic arrest.

Another newly discovered component of the synaptonemal complex, the *FKBP6* gene (located in chromosomal region 7q11.23), was screened in 51 men with non-obstructive azoospermia through direct sequencing methods (Westerveld *et al*., 2005b). Only two heterozygous mutations (T173T and R183C) were identified that are likely to disrupt FKBP6 protein function. However, both mutations were also found in normospermic controls, indicating that one *FKBP6* allele appears to be sufficient for normal spermatogenesis. In conclusion, despite careful selection of patients with relatively homogeneous phenotype similar to that expected based on of mouse model, genetic defects of these two genes are an uncommon cause of azoospermia in humans.

Genes involved in compaction of the sperm nucleus are relevant for correct spermiogenesis. This remarkable repackaging event is related to the requirement for a unique chromatin architecture that would enable a specific transcription schedule after fertilization. Premature translation of *PRM1* mRNA causes precocious nuclear condensation and arrests spermatid differentiation in mice (Lee *et al*., 1995), and the disruption of either the *Prm1* or the *Prm2* gene in mice leads to haploinsufficiency, abnormal chromatin compaction, sperm DNA damage, and male infertility. Although reduction of protamine-2 has been reported in infertile men, mutations in the *PRM2* gene have not been reported in association with reduced PRM2 content (Tanaka *et al*., 2003; Carrell *et al*., 2007). Nishimune and coworkers (Tanaka *et al*., 2003) identified a number of SNP in both the *PRM1* and the *PRM2* genes in a large number of infertile men presenting mainly azoospermia, compared with proven fertile controls. No association between any of the SNP and infertility was observed. However, one mutation in the *PRM2* gene induced a nonsense codon C248T and was present in heterozygosity in one azoospermic patient. Unfortunately, the testis histology of the patient is unknown and this makes it difficult to interpret the consequences of this mutation. In a recent study, a highly selected group of infertile patients was screened for *PRM1* gene mutations (Iguchi *et al*., 2005). A novel SNP, G197T, in a highly conserved region of the gene was identified in 3/30 patients. Based upon the absence of this SNP in over 700 individuals, and the recent confirmatory data from Ravel *et al*. (2007), it appears to be a promising new genetic risk factor for a specific subgroup of infertile patients with abnormal sperm DNA fragmentation and/or teratozoospermia.

Page and collaborators (Wang *et al*., 2001) reported an unexpectedly high number of X-linked genes expressed in the testis. This finding would suggest an important role for X-linked genes in male gametogenesis. However, up to now, only one X-linked gene (*AR*) has been investigated in large-scale studies (see the section on steroid receptor gene mutations), whereas two genes, *USP26* and *TAF7L*, have been screened in relatively small study populations. The *USP26* gene is located on the long arm of the X chromosome (Xq26.2) and belongs to the family of deubiquitinating enzymes that might be involved in histone removal and in the regulation of protein turnover in germ cells. It is specifically expressed in the testis throughout all stages and it was first proposed as having a key function in the early stages of spermatogenesis (Stouffs *et al*., 2005b). Sequence variants have been found mainly in azoospermic men (Paduch *et al*., 2005; Stouffs *et al*., 2005b) and a special cluster of three SNP have been proposed as potential genetic causes for spermatogenic failure. However, the finding of the same cluster in one man with normal spermatogenesis (Stouffs *et al*., 2006a) and its high

frequency in men from sub-Saharan Africa and south and east Asia (Ravel *et al*., 2006) has questioned the pathogenic role of this specific haplotype. For the other X-linked gene *TAF7L*, which is homologous to the autosomal transcription factor gene *TAF7* and is also expressed specifically in the testis, no causative mutations were found in a small study population (Stouffs *et al*., 2006b). However, due to the low number of patients tested, the role of *TAF7L* mutations in spermatogenic failure remains to be established.

Another interesting group of candidate genes are retrogenes. Retrogenes originate from their progenitor genes by retroposition. Several retrogenes reported in recent studies are autosomal, originating from X-linked progenitor genes, and have evolved a testis-specific expression pattern. During male meiosis, sex chromosomes are segregated into a so-called 'XY' body and are silenced transcriptionally. The silencing of the X chromosome during male meiosis could therefore be the driving force behind the retroposition of X-linked genes to autosomes during evolution (Wang, 2004). Despite their potential interest, no large-scale analysis has been performed in testis-specific retrogenes so far.

The Y chromosome: gene mutations and polymorphisms

The long arm of the human Y chromosome hosts a number of genes involved in spermatogenesis, and several types of recurrent Yq deletions are firmly associated with spermatogenic failure (for review see McElreavey and Krausz, 1999; Krausz *et al*., 2003; Skaletsky *et al*., 2003). However, such observations do not allow attribution of spermatogenic function to any particular Y-chromosome-encoded protein because each of these deletions removes multiple genes. Despite the efforts of many research laboratories (results from around 2000 subjects screened for gene-specific deletions have been published) only four cases of confirmed isolated Yq gene mutation/deletion have been reported to date, and all are related to the *AZFa* region (Brown *et al*., 1998; Sun *et al*., 1999; Krausz *et al*., 2006). Since only one laboratory has performed mutation screening in *AZF* genes with direct sequencing (one mutation found out of 560 men), it is difficult to estimate the frequency of point mutations in *AZF* genes.

The rarity of single *AZF* gene deletions is in sharp contrast with the relatively high frequency of *AZF* deletions (classically divided into three azoospermia factor regions: *AZFa*, *AZFb* and *AZFc*; Vogt *et al*., 1996), which represent the most frequent molecular genetic cause of azoospermia and severe oligozoospermia (<5 × 10^6 spermatozoa/ml) (for a review see Krausz *et al*., 2003). A likely explanation is that the peculiar structure and the sequence organization of the Y chromosome make this chromosome prone to the loss of large regions such as the *AZF* gene regions. The complete removal of the *AZFa* and *AZFb* regions is associated with the severe testicular phenotype Sertoli cell-only syndrome, and spermatogenic arrest, respectively. The complete removal of the *AZFc* region causes a variable phenotype, which may range from azoospermia to oligozoospermia. The above-reported genotype/phenotype correlation confers to Y-deletion analysis a prognostic value for testicular sperm retrieval (Krausz and Degl'Innocenti, 2006). The deletion phenotypes suggest that the *AZFa* and *AZFb* regions contain at least one gene with essential spermatogenic function, whereas genes of the *AZFc* region are more likely to affect the efficiency of spermatogenesis.

The *AZFa* region contains two genes, namely *DDX3Y* (former *DBY)* and *USP9Y*, and the removal of both genes is associated with the complete absence of spermatogenic cells in the testis. *USP9Y* is the only Yq gene for which isolated mutations have been reported and no cases of confirmed isolated *DBY* deletions have been reported.

In three cases the exact breakpoint of the deletions has been defined (Sun *et al*., 1999; Krausz *et al*., 2006): the first, a 4-bp splice-donor site deletion resulting in severely truncated protein (Sun *et al*., 1999); the second, the removal of the last 13 exons; and the third, the loss of the promoter and the first 28 exons (Krausz *et al*., 2006). The first patient described by Sun *et al*. (1999) presented azoospermia with a testis histology showing hypospermatogenesis with occasional tubules containing only premeiotic or meiotic germ cells (spermatogenic arrest). This finding led to the conclusion that although complete absence or significant truncation of *USP9Y* protein is not enough to produce the full AZFa phenotype, it still causes azoospermia. In two unique cases of deletions removing part of the *USP9Y* gene, the phenotype was moderate oligoasthenoteratozoospermia and in both cases the mutations were spontaneously transmitted (Krausz *et al*., 2006). In one family the transmission occurred through two generations and represents the first description of a familial case of partial *AZFa* deletion. The second deletion was naturally transmitted from a subfertile father to his son, conceived while the couple were on a waiting list for assisted reproduction techniques. The phenotype of the two last cases clearly indicates that the protein is not required for the acquisition of sperm fertilizing ability, and, during human spermatogenesis, its role is more likely to be a 'fine-tuner', which improves efficiency rather than a provider of essential function. Moreover, it suggests that *DDX3Y* is the most likely candidate for the *AZFa* deletion phenotype.

Apart from the *AZF* genes, the *TSPY* (testis-specific protein, Y-linked) gene may also be involved in spermatogenesis. On the basis of the expression pattern of *TSPY*, the homology of *TSPY* with *SET* (oncoprotein) and the interaction between TSPY and cyclin B Lau (1999) suggests a possible role of TSPY in regulating spermatogonia to enter meiosis. Its role in male infertility remains to be established. It is possible that copy-number variations may influence spermatogenic potential; however, preliminary data showing an association with TSPY dosage by Vodicka *et al*. (2007) needs to be replicated by validated methods and on a large group of patients/controls.

Polymorphisms in the *AZFc* region

Apart from the classical *AZF* deletions, new types of Yq rearrangements have recently attracted the attention of geneticists and andrologists (for review see Noordam and Repping, 2006). The peculiar structure of the *AZFc* region **(Figure 1)** allows a series of rearrangements including the formation of partial deletions, deletion/duplications and partial duplications. Among them the most relevant is termed the 'gr/gr' deletion (Repping *et al*. 2003) and removes half of the *AZFc* content including two copies of the major *AZFc* candidate gene called *DAZ* (Reijo *et al*., 1995). This type of deletion is a significant

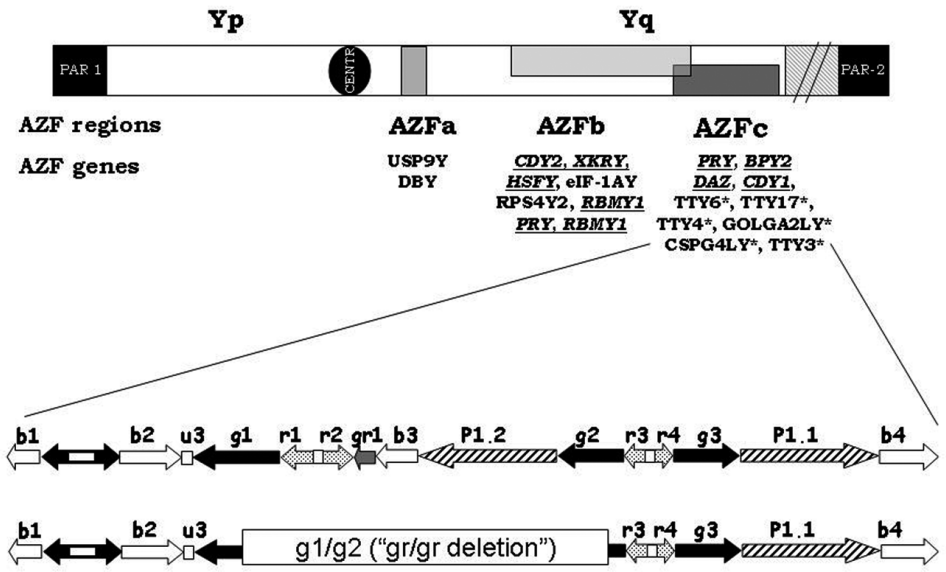

Figure 1. The upper part is a schematic representation of the Y chromosome. Genes of the three azoospermia factor (*AZF*) regions are listed. Deletions of these regions result in spermatogenic failure. Multi-copy genes are in italics, whereas non-italic letters indicate single-copy genes. Underlined genes are expressed exclusively in the testis, whereas non-underlined are expressed ubiquitously. Transcription unit families are indicated with an asterisk. *AZFb* deletions (two subtypes) overlap with the *AZFc* region. Yq = long arm of the Y chromosome; Yp = short arm of the Y chromosome; *PAR* = pseudoautosomal region; *MSY* = male-specific Y with an extension of 63 Mb. The lower part shows the *AZFc* region in detail. This region contains a number of repeated sequences with the same orientation (arrows with the same shading) which through intrachromosomal recombination may lead to deletions. The clinically relevant gr/gr deletion is shown.

risk factor in some populations but apparently not in others (for review see Krausz and Giachini, 2007). Contradictory results are likely to derive from methodological differences together with inappropriate control selection (only in a minority of studies are the controls normospermic men, and controls and patients are not matched for ethnic background in highly mixed populations such as Paris, the south of France and Brazil). Moreover, the majority of studies use sequence-tagged sites plus/minus polymerase chain reaction analysis, which alone does not provide information about the type of missing gene copies and about deletion/duplication events. For this reason the ideal methodology should be a combined analysis based on a first-step sequence-tagged site plus/minus analysis followed by a confirmatory dosage analysis restricted to the samples with suspected deletions, as described by Giachini *et al*. (2005). In the authors' extended series of patients/controls (almost 1000 subjects), after having performed a detailed molecular analysis and compared the frequencies between normospermic and infertile men, the gr/gr deletion proved to be a significant risk factor for abnormal spermatogenesis (Giachini *et al*. 2005; and unpublished data). The gr/gr deletions have been mainly found in oligozoospermic men but in very few cases (2/460) they have also reported in normospermic subjects. Among a number of plausible explanations for the heterogeneous phenotype the following explanations are postulated: (i) The presence of polymorphisms or mutations in the autosomal homologue of the *DAZ* gene *DAZL* (Becherini *et al*., 2004). However, similar to the *AZFc*-deleted patients, no new mutations were found in the entire coding region of the *DAZL* gene except the polymorphic Thr12-Ala change (T12A), which, due to its relatively high frequency in the normospermic group, does not seem to have any modulating effect (Giachini *et al*., 2005); (ii) The type of missing gene copies (*DAZ1*/*DAZ2* and *CDY1A* versus *DAZ3*/*DAZ4* and *CDY1B*); and (iii) The Y background (Y haplogroups). Therefore, it is likely that only a combined analysis will provide a tool for distinguishing between 'neutral' and 'pathogenic' types of deletions.

Recently, Yen and collaborators proposed that not only a reduction but also an excess of *AZFc* gene dosage could be deleterious for spermatogenesis (Lin *et al*., 2007). The authors report that partial gr/gr duplication (with a consequent double dosage of genes situated inside the region) is a significant risk factor for reduced sperm count in the Chinese population. Further studies are needed to define whether gr/gr duplication is a risk factor for reduced sperm count in other populations.

In conclusion, due to the above-mentioned biases (study design, ethnic differences, methodology), it is impossible to perform a reliable meta-analysis of published data on gr/gr deletion. In the few studies with appropriate control selection and confirmation of deletions, gr/gr deletions are clearly a risk factor for spermatogenic disturbances (Repping *et al*., 2003; Giachini *et al*., 2005). Although it is not understood why in certain individuals

the deletion does not affect spermatogenesis, it is likely that Y background (Y haplogroups) plays an important modulating role. In fact, certain Y haplogroups contain constitutive gr/gr deletions and are diffused in certain populations, indicating that other compensatory mechanisms may exist on certain Y backgrounds (Repping *et al*., 2006). Given that fathers bearing gr/gr deletion will obligatorily transmit not only the deletion but also their entire Y chromosome (with or without compensatory structures) to their male offspring, it is likely that their sons will have a similar spermatogenic disturbance in the future. Thus, gr/gr deletion screening should be introduced among genetic diagnostic tests, especially prior to assisted reproduction techniques.

Conclusions

Thanks to the large-scale availability of molecular genetic tools and to the identification of an increasing number of candidate genes, the field of genetics of male infertility is rapidly evolving. However, the advancement in basic research has not been followed by a substantial increase in genetic diagnostic tests. Twelve years on from the description of the three *AZF* regions (Vogt *et al*., 1996), the sole molecular genetic test that is routinely proposed in severe oligo/azoospermic men is the screening for Yq microdeletions. The screening for mutations in genes with predicted specific spermatogenic function has not allowed the identification of clinically relevant mutations. Whether this is due to the real rarity of gene mutations, or to the inappropriateness of the currently used approaches, remains to be established. Recently, Krawetz proposed a new strategy based on sperm transcript profiling that may identify abnormal molecular pathway(s) in specific pathological conditions, such as for example teratozooseprmia (Platts *et al*., 2007). Information obtained from microarray analysis will allow the selection of candidate genes to be screened for mutations in a specific subset of patients with well-defined pathological phenotypes.

Another promising approach, based on the reverse transcription of full length mRNA from spermatozoa, has been proposed by Matzuk and colleagues (Yatsenko *et al*., 2006) and should accelerate the search for clinically relevant mutations. Given the origin of mRNA (haploid germ-cell-expressed genes), the usefulness of both approaches is limited to the detection of genetic defects involved in the post-meiotic maturation of germ cells.

As far as polymorphisms are concerned, the currently used case–control studies are often sources of contradictory results. The lack of reproducibility can be due to different factors already pointed out in the text. A substantial advancement will likely be achieved by the use of new technologies (microarrays) and from multicentre studies (high numbers of well-selected controls and patients of different ethnic origin) able to increase both the efficiency of the screening and the reliability of the results.

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