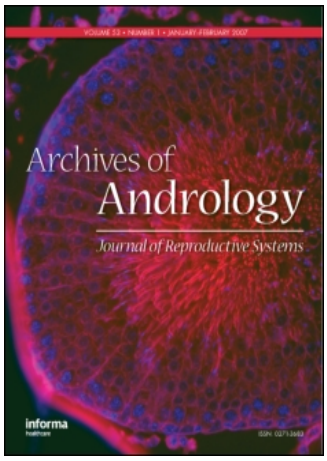
Genetic risk factors in male infertility.



Archives of Andrology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713664683

Genetic Risk Factors in Male Infertility

Online Publication Date: 01 May 2007
To cite this Article: Krausz, Csilla and Giachini, Claudia , (2007) 'Genetic Risk Factors in Male Infertility', Archives of Andrology, 53:3, 125 - 133
To link to this article: DOI: 10.1080/01485010701271786
URL: http://dx.doi.org/10.1080/01485010701271786

Archives of Andrology: Journal of Reproductive Systems, 53:125–133, 2007

ISSN: 0148-5016 print/1521-0375 online
DOI: 10.1080/01485010701271786

informa
healthcare

Review Article

Genetic Risk Factors in Male Infertility

Csilla Krausz
and Claudia Giachini
Andrology Unit, Department of Clinical Physiopathology, Viale Pieraccini, 6, Firenze, Italy

The etiopathogenesis of testicular failure remains unknown in about half of the cases and is referred to as "idiopathic infertility". "Idiopathic" testicular failure is of probable genetic origin since the number of genes involved in human spermatogenesis is likely thousands and only a small proportion of them have been identified and screened in infertile men. In parallel with studies aimed to identify mutations with a clear cause-effect relationship in spermatogenesis candidate genes, there is an increasing interest towards genetic susceptibility factors to male infertility. Despite many efforts, only a few clinically relevant polymorphisms have been identified. This is mainly related to the multifactorial nature of male infertility and to the inappropriate study design of the majority of the studies. The most promising polymorphisms are in genes involved in the endocrine regulation of spermatogenesis and on the Y chromosome, the "gr/gr" deletions. Polymorphisms are generally considered as co-factors. Their final effect on testis function and fertility is probably modulated by the genetic background of each individual and/or by the presence of certain environmental factors. In this review, recent findings concerning some of the most widely studied polymorphisms and male infertility will be discussed.

KEYWORDS gene mutations, genetic risk factors, genetics, gr/gr deletions, male infertility, polymorphisms, spermatogenesis

INTRODUCTION

There is an increased interest in using genetic markers like polymorphisms in all areas of medicine including male infertility. A genetic variant may be directly responsible for a given phenotype if it affects the function or expression of a protein or if it can be in linkage disequilibrium with a functionally relevant mutation in the same or in another related gene. Therefore, studies dealing with polymorphisms are not only important for identifying genetic "risk factors," but they may also represent an important starting point for searching for genes involved in a given disease through linkage analysis. The identification of functionally relevant polymorphisms are also important from a pharmacogenomic point of view and will be probably used in the future for personalized therapies. In this review recent findings concerning some of the most extensively studied polymorphisms and male infertility will be discussed.

Abbreviations: AR: Androgenic Receptor; ERS: Estrogenic Receptor; FSHR: Follicle-Stimulating Hormone Receptor; TA; SNP12

Received 25 January 2007; accepted 09 February 2007.

Address correspondence to Csilla Krausz, Andrology Unit, Department of Clinical Physiopathology, Viale Pieraccini, 6, 50139 Firenze, Italy. E-mail: c.krausz@dfc.unifi.it

TABLE 1 **Summary of Case Control Association Studies of Different Polymorphisms in Genes or in DNA Sequences with Potential Effect on Spermatogenesis**

Case Control Studies		
Polymorphism	More than one study	Single study
▪ in **GENES** involved in:		
Endocrine regulation of spermatogenesis	AR FSHR ESR1 ESR2	CYP19A1 NRIP1
Specific spermatogenic functions	DAZL PRM1–PRM2 TNP1–TNP2 USP26	GRTH CREM–ACT KIT–KITLG
Common cell functions (metabolism, cell cycle, mutation repair)	POLG MTHFR	GSTM1 PHGPx BRCA2 MS MTRR APOB
▪ in **DNA sequences**:	Y-chromosome haplogroups ''gr/gr'' deletions	Mitochondrial DNA haplogroups

Abbreviations: 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**: Estrogen Receptor; **FSHR:** Follicle-Stimulating Hormone Receptor; **GRTH:** Gonadotrophin-Regulated Testicular Helicase; **GSTM1:** Glutathione S-Transferase M1; **KIT:** v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; **KITLG:** KIT Ligand; **MS:** methionine synthase; **MTHFR:** 5,10-MethyleneTetraHydroFolate Reductase; **GSTM1:** Glutathione S-transferase M1; **PHGPx:** Phospholipid Hydroperoxide Glutathione Peroxidase; **MTRR:** MS Reductase; **NRIP:** nuclear receptor interacting protein; **POLG:** mitochondrial DNA gamma-polymerase; **PRM:** Protamine; **TNP:** Transition Nuclear Protein; **USP26:** Ubiquitin Specific Peptidase 26.

Until now, the most widely used approach for the study of haplogroups, allele variants, and single nucleotide polymorphisms in male infertility were based on case control studies. For many genes only sporadic data are available, or, when more studies are published on the same polymorphism results are often contradictory (Table 1). The question arises, how do we resolve this sometimes seemingly contradictory data?

POLYMORPHISMS IN GENES INVOLVED IN THE ENDOCRINE REGULATION OF SPERMATOGENESIS

The crucial role of androgens, gonadotropins and estrogens in the endocrine regulation of spermatogenesis is well known, thus the genes of their receptors represent a logical target for mutational analysis in the infertile male.

The androgen receptor (AR) is a ligand activated transcription factor which is encoded by the AR gene located on the long arm of the X chromosome (Xq11-q12). The AR gene has been the object of a large quantity of studies, and both mutation screenings of the entire coding sequence and the promoter region have been reported [Yong et al. 2003]. The first exon of the AR codes for the transactivation regulating domain and contains two polymorphic tracts, a CAG and a GGC repeat sequence. The polymorphic (CAG)n codes for polyglutamine whereas the (GGC)n repeat for polyglicine. It has been demonstrated *in vitro* for the (CAG)n repeats 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 ARs with GGN repeat, lengths other than the most common one of 23, have a lower transactivating capacity [Lundin et al. 2007].

As expected from early *in vitro* studies, CAG repeat length and male infertility have shown a significant association between relatively long CAG repeats and impaired sperm production. However, subsequent

studies gave rather contradictory results. This in part can be the consequence of ethnic differences (the association seems to be more consistent in the Asiatic populations) although the heterogenicity of the control (unselected men or proven fertile men or normospermic men) and of the infertile (different inclusion criteria) groups [for review see Krausz et al. 2004a and references therein] may also play an important role [for review see Asatiani et al. 2003 and references therein].

Similarly, there is no clear agreement about the effect of GGC repeat length and sperm production. Recently, two European groups attempted to evaluate the joint effect of both exon 1 polymorphic microsatellites on male infertility [Ferlin et al. 2004; Ruhayel et al. 2004], but the "protective" and "at risk" CAG/GGC haplotypes were different in the two Caucasian populations. Due to these discordant association data and to the lack of *in vitro* expression studies on the effect of varying GGC length in combination with different CAG repeats, the clinical utility of the CAG/GGC haplotype definition remains unclear.

In summary, if only data based on large study populations are considered, the CAG repeat length polymorphism is an unlikely risk factor for male infertility. However, its role in modulating androgen action is evident in patients affected by Klinefelter's syndrome [Lanfranco et al. 2004], in hypogonadal men undergoing T replacement therapy [Zitzmann et al. 2004] and in hypoandrogenic males [Canale et al. 2005]. It is therefore possible that the mild functional effect of a long polyglutamine stretch can be compensated by a relatively high serum testosterone level, ergo the polymorphism should not be evaluated in isolation but always in the context of environmental factors. Moreover, the analysis of CAG repeat length could be useful for personalized substitutive testosterone therapy.

Although the physiological role of estrogens in spermatogenesis is not clearly defined, human and animal models exhibit an association between estrogen insufficiency and abnormal spermatogenesis. While recent studies suggest a role as a survival factor [Pentikainen et al. 2000], the excess of this hormone during the neonatal period or adulthood can impair sperm production in rats [Atanassova et al. 2000]. The physiological responses to estrogens are known to be mediated by at least two functional isoforms of estrogen receptors (ER), i.e., ERalpha (ESR1) and ERbeta (ESR2), encoded by two different genes on different chromosomes (6q25 and 14q23-24, respectively). Apart from estradiol, other compounds with estrogen-like activity (xenoestrogens) may bind to ERs and may account for the reported decline in sperm count as well as for the increased incidence of other components of the testicular dysgenesis syndrome—hypospadias, cryptorchidism, and testicular cancer—observed in the last 50 years [Sharpe and Skakkebaek 1993].

Genetic screening of the ESR1 and ESR2 genes has revealed the existence of several polymorphic sites in both genes and some have been the object of male infertility association studies [Kukuvitis et al. 2002; Suzuki et al. 2002; Galan et al. 2005; Guarducci et al. 2006]. In the ESR1 gene, the most promising polymorphism is the $(TA)_n$ variable number of tandem repeats (VNTR) within the promoter region. The distribution of TA genotype is not different between controls and patients. This polymorphism cannot be considered a risk factor for male infertility. However, it has a significant effect on sperm output both in normospemic 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 length on both alleles have significantly lower sperm production. Since previous studies on lumbar bone mineral density observed that allelic combinations with a higher number of TA repeats are functionally more active [Becherini et al. 2000], our finding indicates that allelic combinations which confer a stronger estrogen effect may negatively influence human spermatogenesis. A plausible explanation would be that in addition to a deficit of estrogens there is an exaggerated estrogen action related to this genetic variant, that when combined with environmental factors, can be deleterious. Whether the observed negative effect reflects the expression of a disturbance in early testis development or in the adult testis and whether it is related to xenoestrogens remain to be established.

A specific haplotype AGATA, resulting from the allelic combination of five SNPs situated in a 50 Kb haplotype block of the ESR1, has been reported as a risk factor for cryptorchidism in the Japanese population [Yoshida et al. 2005]. We analyzed the AGATA haplotype in the Spanish and Italian infertile and control populations and found no association between SNP12 (the tag SNP for the AGATA haplotype) and infertility [Galan et al. 2007].

Surprisingly, we found a significant protective effect for ESR1 SNP12 on cryptorchidism in the Italian population. The discrepancy between our studies may be related to genuine ethnic differences and/or different environmental conditions.

To date, only two association studies evaluated the role of ESR2 gene SNPs in male infertility [Aschim et al. 2005; Galan et al. 2005]. In one study the frequency of the heterozygous RsaI AG-genotype was three times higher in infertile men than in controls, indicating that this polymorphism may have modulating effects on spermatogenesis [Aschim et al. 2005]. In the other study by Galan et al. [2005] no significant association was found between another ESR2 SNP and infertility. However, the authors detected genetic interaction between five estrogen related gene markers identifying a set of protective predisposing haplotypes.

In summary, preliminary data suggest that ESR1 and ESR2 polymorphisms may influence male fertility, spermatogenic efficiency, and cryptorchidism. It will be of interest to verify the effect of these ER polymorphisms on spermatogenic potential in a selected group of subjects with different grades of exposure to xenoestrogens.

Follicle-stimulating hormone (FSH) the specific receptor (FSHR) consists of 10 exons, also plays an important role in spermatogenisis located on chromosome 2 (2p21-p16). Mutation screening of the FSHR gene revealed three relevant single nucleotide polymorphisms (SNPs), the SNP in the core promoter at position −29 and two others situated in exon 10 that correspond to amino acid position 307 and 680, respectively [for review see Gromoll and Simoni 2005]. A study of the distribution of the SNPs in normal and infertile men reported that the combination of the exon 10 SNPs with the − 29 SNP can be considered as a new genetic factor for severe spermatogenic impairment [Ahda et al. 2005]. However, a recent report on the Italian population was not able to identify a predisposing effect of the combined exon 10 SNPs and the −29 SNP [Pengo et al. 2006]. Given these discordances further studies are needed to clarify this issue.

EXAMPLE OF POLYMORPHISMS IN CANDIDATE AUTOSOMAL SPERMATOGENESIS GENES

A number of spermatogenesis autosomal candidate genes have been identified and they represent the most obvious targets for mutation analysis [Stouffs et al. 2005; A et al. 2006; Galan et al. 2006]. As reported in Table 1, results are often contradictory when more than one study is available. A remarkable ethnic difference has been found for the T54A DAZL gene polymorphism. The DAZL gene is an autosomal homologue of the Y chromosomal DAZ (deleted in azoospermia) gene cluster and is located on chromosome 3p24 [Yen et al. 1996]. DAZ and DAZL, together with BOULE, are members of the same family and encode RNA binding proteins with an important role in spermatogenesis [Yen 2004]. The T54A variant in the DAZL gene was reported as a susceptibility factor to oligo/azoospermia in the Chinese population [Teng et al. 2002]. This SNP is localized within the highly conserved RNA-recognition motif domain of the DAZL protein and it may lead to functional consequences such as reduced RNA binding. Despite this promising finding, subsequent studies in Caucasian populations [Becherini et al. 2004; Tschanter et al. 2004; Bartoloni et al. 2004] and in the Japanese population [Yang et al. 2005] failed to detect the T54A mutation in more than 900 men. This strongly contrasts the relatively high frequency of this mutation (7.4%) in the Chinese patients.

The Protamine1 and 2 (PRM1 and PRM2) genes were the object of several studies [Tanaka et al. 2003; Miyagawa et al. 2005; Iguchi et al. 2006; Aoki et al. 2006] which finally lead to the conclusion that PRM1 polymorphism is probably relevant in a specific subset of patients. Since aberrations in the ratio PRM1/PRM2 are associated with human male infertility [for references see Aoki et al. 2006] it is expected that the ratio of anomalies can be related to mutations in genes involved in the chromatin compaction such as PRM1, PRM2, TNP1 and TNP2. According to this hypothesis, Carrell and his group [Aoki et al. 2006] screened for alterations in the genes encoding PRM1, PRM2, TNP1 and TNP2 in a population of idiopathic infertile men and a highly selected group of patients with aberrations in the PRM1/PRM2 ratio. The frequency of the 15 identified SNPs was similar in the protamine-deficient patients, severely infertile patient and fertile controls, indicating an unlikely role for these variants for protamine deficiency. A study on normospermic men with high sperm DNA fragmentation (similar to the sperm derived from protamine deficient mice) found a mutation G197T in the PRM-1 gene which was specific for this highly selected group of subjects [Iguchi et al. 2006].

Products of the CREM gene (on chromosome 10p11.1–12.1) are essential for the differentiation of round spermatids into mature spermatozoa [Sassone-Corsi 1995; Blendy et al. 1996; Nantel et al. 1996; Krausz and Sassone-Corsi 2005] and they are regulated in part by an activator of CREM in the testis, ACT (6q16.1–16.3) which is expressed exclusively in the testis. Both genes were objects of mutation screening. CREM was analyzed in a specific group of men with round spermatid arrest [Vouk et al. 2005]. In this pilot study a number of genetic changes have been identified and it seems that certain patterns of homozygous and heterozygous alterations could exert pathological effects. A recent mutational screening in the ACT gene reports specific ACT haplotypes which show statistically significant differences between patients and controls suggesting a potential effect of different allelic combinations on spermatogenesis [Christensen et al. 2006].

Screening of a larger number of patients and controls is required to elucidate whether the observed combinations of genetic changes in the CREM and ACT genes can reconcile some forms of male infertility.

EXAMPLES OF POLYMORPHISMS IN GENES WITH COMMON CELL FUNCTIONS

Studies of polymorphisms in genes involved in common cell functions required for normal spermatogenesis [Zhoucun et al. 2006; Peterlin et al. 2006; please see other references through text] are summarized in Table 1. Among them, the role of mitochondrial DNA polymerase γ (POLG) has been the object of extensive debate in the last few years. The mitochondrial DNA polymerase γ (POLG) is the sole polymerase for mitochondrial DNA (mtDNA) and impaired activity of this protein leads to mitochondrial dysfunction through accumulation of mtDNA mutations. The gene maps to 15q24–15q26 and its first exon contains a polyglutamine tract encoded by a motif $(CAG)_{10}$ CAACAGCAG [Ropp and Copeland 1996]. The length of the CAG repeat is polymorphic with a major allele at 10 repeats. Rovio and colleagues [2001] proposed an association between the absence of the common 10 CAG allele and male infertility in a relatively small group of infertile (n = 99) and fertile (n = 98) men. Another study on the Danish population observed a significantly higher frequency of homozygous not10 CAG repeat allele in a subgroup of men affected by unexplained infertility, i.e. normal sperm count, motility, and morphology [Jensen et al. 2004]. However, this conclusion was based on an interpretation bias i.e., the seven unexplained infertile men with the homozygous not10/not10 CAG genotype were not normospermic, with the exception of one subject. When appropriately calculated the frequency is 1/42 (2.38%) not 7/49 (14.3%) the no significant difference with respect to the control fertile group (0.8%). Our own study on the Italian population (n = 385) found no relationship between the polymorphic CAG repeat in the POLG gene and idiopathic male infertility [Krausz et al. 2004b]. Subsequent studies in populations from Europe [Aknin-Seifer et al. 2005; Brusco et al. 2006] and New Zeland [Harris et al. 2006] further confirmed our finding.

It is therefore clear that the POLG CAG polymorphism has no clinical significance for idiopathic or "unexplained" male infertility. It should also be clarified if the length of the CAG tract has any functional effect on the polymerase activity. This is required before we can propose it as a possible genetic factor for asthenozoospermia.

Some of the genes listed in Table 1 may have a role in specific conditions. For example polymorphisms in the glutathione S-transferase M1 (GSTM1) and Phospholipid hydroperoxide glutathione peroxidase (PHGPx) may be relevant under conditions of oxidative stress (for example varicocele) [Chen et al. 2002; Maiorino et al. 2003]. Similarly, another gene polymorphism C677T in the methylenetetrahydrofolate reductase (MTHFR) gene seems to have clinical relevance only in specific environmental conditions which are more common in Indian, African, and Southeast Asian populations [Bezold et al. 2001; Ebisch et al. 2003; Stuppia et al. 2003; Singh et al. 2005; Park et al. 2005] characterized by low dietary intake of folates. Two promising SNPs in genes involved in the folate metabolism were also proposed recently in the Korean population [Lee et al. 2006]. Their role in other geographic areas remains to be established.

Y CHROMOSOME POLYMORPHISMS: THE "ggr/gr" DELETIONS

Apart from the classical AZF deletions, a new type of Yq deletion has recently attracted the attention of

TABLE 2 Summary of ''gr/gr'' Deletion Case Control Studies with or without Association with Spermatogenic Disturbances

		Patients		Controls			
Reference	Population	Tot n.	gr/gr %	Tot n.	Normospermic %	gr/gr %	Gene Dosage
Positive Association							
Repping et al. (2003)	Dutch	246	**3.7**	148	100	**0.0**	yes
de Llanos et al. (2005)	Spanish	283	**4.2**	232	14.6	**0.0**	no
Ferlin et al. (2005)	Italian	337	**5.3**	263	100	**0.4**	no
Giachini et al. (2005)	Italian	150	**5.3**	189	100	**0.5**	yes
Lynch et al. (2005)	Australian	1351	**4.1**	234	57.3	**0.4**	no
No Association							
Machev et al. (2004)	French	300	**6.0**	399	1.2	**3.5**	yes
Hucklenbroich et al. (2005)	German	348	**4.0**	170	100	**1.8**	no
Ravel et al. (2006)	Mixed	192	**2.1**	181	8.8	**3.3**	no
Carvalho et al. (2006)	Brazilian	110	**4.5**	240	0.0	**2.9**	no

Ethnic/geographic origin and the size of the study population is reported. Since the inclusion criteria for controls was different in different studies (general population or proven fertile men with unknown sperm count, normospemic men), the percentage of normospermic men in each control group is indicated. The frequency of ''gr/gr'' deletions in patients and controls are shown separately. Gene dosage was performed in only 3/9 studies in order to confirm the deletions and exclude deletion/duplication events.

geneticists and andrologists. A partial deletion in the AZFc region, termed "gr/gr" has been described specifically in infertile men with varying degrees of spermatogenic failure [Repping et al. 2003]. This deletion removes half the AZFc gene content including two copies of the major AZFc candidate gene called DAZ [Reijo et al. 1995; Vogt et al. 2004]. In the last two years an intensive search for "gr/gr" deletions in infertile and control men has started in order to define their frequency and clinical significance [Machev et al. 2004; de Llanos et al. 2005; Ferlin et al. 2005; Giachini et al. 2005; Hucklenbroich et al. 2005; Lynch et al. 2005; Ravel et al. 2006; Carvalho et al. 2006]. Some of these studies reached the conclusion that "gr/gr" deletions are significant risk factors for oligo/azoospermia while other studies did not find such an effect (Table 2).

The current method for the detection of "gr/gr" deletions is based on STS plus/minus PCR analysis. This alone does not provide information about the type of missing gene copies or deletion/duplication events. The majority of "gr/gr" studies are lacking detailed molecular analysis, i.e., the reduced gene dosage is not confirmed and the type of deleted gene copies is not known. These methodological differences together with the inappropriate control selection (general population or fertile controls with unknown sperm count) may contribute to the contradictory results.

In contrast to the classical AZF deletions, "gr/gr" deletions can also be found in normospermic men. We hypothesized a number of explanations for the heterogeneous phenotype ranging from severe spermatogenic failure to normozoospermia: i) presence of polymorphisms or mutations in the autosomal homologue of the DAZ gene, DAZL; ii) deletion/duplication events or other rearrangements that could maintain the original gene dosage and consequently would not affect spermatogenesis; iii) differences in the type of deleted AZFc gene copies and iv) different Y chromosome background. In order to verify these hypotheses, an accurate molecular characterization of the deletions and DAZL gene mutation screening was performed in our laboratory [Giachini et al. 2005]. Our studies excluded a role for DAZL gene mutations in the "gr/gr" phenotype, whereas the effect and the frequency of deletion/duplication events remains to be established. The definition of the missing DAZ (1/2 or 3/4) and CDY1 (a or b) copies allowed us to divide "gr/gr" deletions into different subtypes; two of them, which remove CDY1a copy are specific and highly frequent in patients, indicating that certain subtypes are more pathogenic than others.

Consequently, a combined molecular characterization (haplogroup, gene dosage, and gene copy type definition) of the "gr/gr" deleted patients and controls will probably allow the distinction between pathogenic and neutral deletions. Unfortunately, it is impossible to perform a metaanalysis of the published data due to methodological differences and

different inclusion criteria of the controls. However, on the basis of our own data from almost 800 subjects, we can safely conclude that "gr/gr" deletions are a highly significant risk factor ($p < 0.001$) for oligozoospermia. Consequently, the screening for "gr/gr" deletions can be advised to patients attending for assisted reproductive techniques, since this test is able to provide the identification of a transmissible genetic risk factor for reduced sperm count.

CONCLUSIONS

Despite a generalized enthusiasm towards the analysis of polymorphisms in genes affecting spermatogenesis, to date, only a few clinically relevant polymorphisms have been identified. We often face frustration when initial promising data are not confirmed in later studies. Discrepancies between association studies are rather frequent and mainly related to small sample size (especially when subjects are further divided into subgroups according to different allelic combinations) and to the use of inappropriate control groups. In the majority of studies, controls are subjects from the general population or selected only on the basis of their fertility with unknown sperm parameters. If the expected effect of a polymorphism is spermatogenic disturbance, the correct control group should be normospermic men. If the polymorphism is predicted to influence the fertilization capacity, the most appropriate control should be proven fertile men. The most evident examples supporting the importance of normospemic controls and of the appropriate size of the study population are "gr/gr" deletions and the POLG studies. Selection of controls along with the appropriate "cases" are crucial, especially if the gene defect is expected to lead to a specific testicular phenotype. Certain gene variants may cause specific phenotypes (for example PRM1 and CREM) and consequently it can be expected that only the analysis of a specific subgroup of patients will be able to identify their clinical significance.

Apart from the sample size and selection bias, genuine ethnic and geographic differences can also contribute to the lack of confirmation of results in different populations. The recently described DAZL gene polymorphism or the SNP12 in ESR1 represent remarkable examples of ethnic differences [Becherini et al. 2004; Galan et al. 2006].

Since polymorphisms are generally considered as risk factors, their final effect on spermatogenesis is probably modulated by additional factors such as genetic background or environment. Considering the multitude of factors and biases which can affect the outcome of a case/control study, it is not surprising that published data are so contradictory. Large scale multicenter and multiethnic studies with multiple markers (using a complex trait model) are needed in order to identify reliable risk factors for spermatogenic disturbances or for male infertility.

REFERENCES

Ahda, Y., Gromoll, J., Wunsch, A., Asatiani, K., Zitzmann, M., Nieschlag, E. and Simoni, M. (2005) Follicle-stimulating hormone receptor gene haplotype distribution in normozoospermic and azoospermic men. J Androl **26**:494–499.

Aknin-Seifer, I. E., Touraine, R. L., Lejeune, H., Jimenez, C., Chouteau, J., Siffroi, J. P., McElreavey, K., Bienvenu, T., Patrat, C. and Levy, R. (2005) Is the CAG repeat of mitochondrial DNA polymerase gamma (POLG) associated with male infertility? A multi-centre French study. Hum Reprod **20**:736–740.

Aoki, V. W., Christensen, G. L., Atkins, J. F. and Carrell, D. T. (2006) Identification of novel polymorphisms in the nuclear protein genes and their relationship with human sperm protamine deficiency and severe male infertility. Fertil Steril **86**:1416–1422.

Asatiani, K., von Eckardstein, S., Simoni, M., Gromoll, J. and Nieschlag, E. (2003) CAG repeat length in the androgen receptor gene affects the risk of male infertility. Int J Androl **26**:255–261.

Aschim, E. L., Giwercman, A., Stahl, O., Eberhard, J., Cwikiel, M., Nordenskjold, A., Haugen, T. B., Grotmol, T. and Giwercman, Y. L. (2005) The RsaI polymorphism in the estrogen receptor-beta gene is associated with male infertility. J Clin Endocrinol Metab **90**: 5343–5348.

Atanassova, N., McKinnell, C., Turner, K. J., Walker, M., Fisher, J. S., Morley, M., Millar, M. R., Groome, N. P. and Sharpe, R. M. (2000) Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: Evidence for stimulatory effects of low estrogen levels. Endocrinology **141**:3898–3907.

Bartoloni, L., Cazzadore, C., Ferlin, A., Grolla, A. and Foresta, C. (2004) Lack of the T54A polymorphism of the DAZL gene in infertile Italian patients. Mol Hum Reprod **10**:613–615.

Becherini, L., Gennari, L., Masi, L., Mansani, R., Massart, F., Morelli, A., Falchetti, A., Gonnelli, S., Fiorelli, G., Tanini, A. and Brandi, M. L. (2000) Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor alpha gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Genet **9**:2043–2050.

Becherini, L., Guarducci, E., Degl'Innocenti, S., Rotondi, M., Forti, G. and Krausz, C. (2004) DAZL polymorphisms and susceptibility to spermatogenic failure: An example of remarkable ethnic differences. Int J Androl **27**:375–381.

Bezold, G., Lange, M. and Peter, R. U. (2001) Homozygous methylenetetrahydrofolate reductase C677T mutation and male infertility. N Engl J Med **344**:1172–1173.

Blendy, J. A., Kaestner, K. H., Weinbauer, G. F., Nieschlag, E. and Schutz, G. (1996) Severe impairment of spermatogenesis in mice lacking the CREM gene. Nature **380**:162–165.

Brusco, A., Michielotto, C., Gatta, V., Foresta, C., Matullo, G., Zeviani, M., Ferrari, G., Dragone, E., Calabrese, G., Rossato, et al. (2006) The polymorphic polyglutamine repeat in the mitochondrial DNA

polymerase gamma gene is not associated with oligozoospermia. J Endocrinol Invest **29**:1–4.

Canale, D., Caglieresi, C., Meschini, C., Liberati, C. D., Macchia, E., Pinchera, A. and Martino, E. (2005) Androgen receptor polymorphism (CAG repeats) and androgenicity. Clin Endocrinol **63**:356–361.

Carvalho, C. M., Zuccherato, L. W., Bastos-Rodrigues, L., Santos, F. R. and Pena, S. D. (2006) No association found between gr/gr deletions and infertility in Brazilian males. Mol Hum Reprod **12**:269–273.

Chen, S. S., Chang, L. S., Chen, H. W. and Wei, Y. H. (2002) Polymorphisms of glutathione S-transferase M1 and male infertility in Taiwanese patients with varicocele. Hum Reprod **17**:718–725.

Christensen, G. L., Wooding, S. P., Ivanov, I. P., Atkins, J. F. and Carrell, D. T. (2006) Sequencing and haplotype analysis of the activator of CREM in the testis (ACT) gene in populations of fertile and infertile males. Mol Hum Reprod **12**:257–262.

de Llanos, M., Ballesca, J. L., Gazquez, C., Margarit, E. and Oliva, R. (2005) High frequency of gr/gr chromosome Y deletions in consecutive oligospermic ICS candidates. Hum Reprod **20**:216–220.

Ebisch, I. M., van Heerde, W. L., Thomas, C. M., van der Put, N., Wong, W. Y. and Steegers-Theunissen, R. P. (2003) C677T methylenetetrahydrofolate reductase polymorphism interferes with the effects of folic acid and zinc sulfate on sperm concentration. Fertil Steril **80**:1190–1194.

Ferlin, A., Tessari, A., Ganz, F., Marchina, E., Barlati, S., Garolla, A., Engl, B. and Foresta, C. (2005) Association of partial AZFc region deletions with spermatogenic impairment and male infertility. J Med Genet **42**:209–213.

Ferlin, A., Bartoloni, L., Rizzo, G., Roverato, A., Garolla, A. and Foresta, C. (2004) Androgen receptor gene CAG and GGC repeat lengths in idiopathic male infertility. Mol Hum Reprod **10**:417–421.

Galan, J. J., Buch, B., Cruz, N., Segura, A., Moron, F. J., Bassas, L., Martinez-Pineiro, L., Real, L. M. and Ruiz, A. (2005) Multilocus analyses of estrogen-related genes reveal involvement of the ESR1 gene in male infertility and the polygenic nature of the pathology. Fertil Steril **84**:910–918.

Galan, J. J., De Felici, M., Buch, B., Rivero, M. C., Segua, A., Royo, J. L., Cruz, N., Real, L. M. and Ruiz, A. (2006) Association of genetic markers within the KIT and KITLG genes with human male infertility. Hum Reprod **21**:3185–3192.

Galan, J. J., Guarducci, E., Nuti, F., Gonzalez, A., Ruiz, M., Ruiz, A. and Krausz, C. (2007) Molecular analysis of estrogen receptor alpha gene AGATA haplotype and SNP12 in European populations: Potential protective effect for cryptorchidism and lack of association with male infertility. Hum Reprod **22**:444–449.

Giachini, C., Guarducci, E., Longepied, G., Degl'Innocenti, S., Becherini, L., Forti, G., Mitchell, M. J. and Krausz, C. (2005) The gr/gr deletion(s): A new genetic test in male infertility? J Med Genet **42**:497–502.

Gromoll, J. and Simoni, M. (2005) Genetic complexity of FSH receptor function. Trends Endocrinol Metab **16**:368–373.

Guarducci, E., Nuti, F., Becherini, L., Rotondi, M., Balercia, G., Forti, G. and Krausz, C. (2006) Estrogen receptor {alpha} promoter polymorphism: stronger estrogen action is coupled with lower sperm count. Hum Reprod **21**:994–1001.

Harris, T. P., Gomas, K. P., Weir, F., Holyoake, A. J., McHugh, P., Wu, M., Sin, Y., Sin, I. L. and Sin, F. Y. (2006) Molecular analysis of polymerase gamma gene and mitochondrial polymorphism in fertile and subfertile men. Int J Androl **29**:421–433.

Hucklenbroich, K., Gromoll, J., Heinrich, M., Hohoff, C., Nieschlag, E. and Simoni, M. (2005) Partial deletions in the AZFc region of the Y chromosome occur in men with impaired as well as normal spermatogenesis. Hum Reprod **20**:191–197.

Iguchi, N., Yang, S., Lamb, D. J. and Hecht, N. B. (2006) An SNP in protamine 1: A possible genetic cause of male infertility? J Med Genet **43**:382–384.

Jensen, M., Leffers, H., Petersen, J. H., Nyboe Andersen, A., Jorgensen, N., Carlsen, E., Jensen, T. K., Skakkebaek, N. E. and Rajpert-De Meyts, E. (2004) Frequent polymorphism of the mitochondrial DNA polymerase gamma gene (POLG) in patients with normal spermiograms and unexplained subfertility. Hum Reprod **19**:65–70.

Krausz, C., Quintana-Murci, L. and Forti, G. (2004a) Y chromosome polymorphisms in medicine. Ann Med **36**:573–583.

Krausz, C., Guarducci, E., Becherini, L., Degl'Innocenti, S., Gerace, L., Balercia, G. and Forti, G. (2004b) The clinical significance of the POLG gene polymorphism in male infertility. J Clin Endocrinol Metab **89**:4292–4297.

Krausz, C. and Sassone-Corsi, P. (2005) Genetic control of spermiogenesis: insights from the CREM gene and implications for human infertility. Reprod Biomed Online **10**:64–71.

Kukuvitis, A., Georgiou, I., Bouba, I., Tsirka, A., Giannouli, C. H., Yapijakis, C., Tarlatzis, B., Bontis, J., Lolis, D., Sofikitis, N. and Papadimas, J. (2002) Association of oestrogen receptor alpha polymorphisms and androgen receptor CAG trinucleotide repeats with male infertility: A study in 109 Greek infertile men. Int J Androl **25**:149–152.

Lanfranco, F., Kamischke, A., Zitzmann, M. and Nieschlag, E. (2004) Klinefelter's syndrome. Lancet **364**:273–283.

Lee, H. C., Jeong, Y. M., Lee, S. H., Cha, K. Y., Song, S. H., Kim, N. K., Lee, K. W. and Lee, S. (2006) Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility. Hum Reprod **21**:3162–3170.

Lundin, K. B., Giwercman, A., Dizeyi, N. and Giwercman, Y. L. (2007) Functional in vitro characterisation of the androgen receptor GGN polymorphism. Mol Cell Endocrinol **264**:184–187.

Lynch, M., Cram, D. S., Reilly, A., O'Bryan, M. K., Baker, H. W., de Kretser, D. M. and McLachlan, R. I. (2005) The Y chromosome gr/gr gr subdeletion is associated with male infertility. Mol Hum Reprod **11**:507–512.

Machev N., Saut N., Longepied G., Terriou P., Navarro A., Levy N., Guichaoua M., Metzler-Guillemain C., Collignon P., Frances A-M., et al. (2004) Sequence family variant loss from the AZFc interval of the human Y chromosome, but not gene copy loss, is strongly associated with male infertility. J Med Genet **41**:814–825.

Maiorino, M., Bosello, V., Ursini, F., Foresta, C., Garolla, A., Scapin, M., Sztajer, H. and Flohe, L. (2003) Genetic variations of gpx-4 and male infertility in humans. Biol Reprod **68**:1134–1141.

Miyagawa, Y., Nishimura, H., Tsujimura, A., Matsuoka, Y., Matsumiya, K., Okuyama, A., Nishimune, Y. and Tanaka, H. (2005) Single-nucleotide polymorphisms and mutation analyses of the TNP1 and TNP2 genes of fertile and infertile human male populations. J Androl **26**:779–786.

Nantel, F., Monaco, L., Foulkes, N. S., Masquilier, D., LeMeur, M., Henriksen, K., Dierich, A., Parvinen, M. and Sassone-Corsi, P. (1996) Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. Nature **380**:159–162.

Park, J. H., Lee, H. C., Jeong, Y. M., Chung, T. G., Kim, H. J., Kim, N. K., Lee, S. H. and Lee, S. (2005) MTHFR C677T polymorphism associates with unexplained infertile male factors. J Assit Reprod Genet **22**:361–368.

Pengo, M., Ferlin, A., Arredi, B., Ganz, F., Selice, R., Grolla, A. and Foresta, C. (2006) FSH receptor gene polymorphisms in fertile and infertile Italian men. Reprod Biomed Online **13**:795–800.

Pentikainen, V., Erkkila, K., Suomalainen, L., Parvinen, M. and Dunkel, L. (2000) Estradiol acts as a germ cell survival factor in the human testis in vitro. J Clin Endocrinol Metab **85**:2057–2067.

Peterlin, B., Zorn, B., Volk, M. and Kunej, T. (2006) Association between the apolipoprotein B signal peptide gene insertion/deletion polymorphism and male infertility. Mol Hum Reprod **12**:777–779.

Ravel, C., Chantot-Bastaraud, S., El Houate, B., Mandelbaum, J., Siffroi, J. P. and McElreavey, K. (2006) gr/gr deletions within the azoospermia factor c region on the Y chromosome might not be associated with spermatogenic failure. Fertil Steril **85**:229–231.

Reijo, R. A., Lee, T. Y., Salo, P., Alagappan, R., Brown, L. G., Rosenberg, M., Rozen, S., Jaffe, T., Straus, D., Hovatta, et al. (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet **10**: 383–393.

Repping, S., Skaletsky, H., Brown, L., Van Daalen, S. K., Korver, C. M., Pyntikova, T., Kuroda-Kawaguchi, T., De Vries, J. W., Oates, R. D., Silber, S., et al. (2003) Polymorphism for a 1.6-Mb deletion of the human Y chromosome persists through balance between recurrent mutation and haploid selection. Nat Genet **35**:247–251.

Ropp, P. A. and Copeland, W. C. (1996) Cloning and characterization of the human mitochondrial DNA polymerase, DNA polymerase gamma. Genomics **36**:449–458.

Rovio, A. T., Marchington, D. R., Donat, S., Schuppe, H. C., Abel, J., Fritsche, E., Elliott, D. J., Laippala, P., Ahola, A. L., McNay, et al. (2001) Mutations at the mitochondrial DNA polymerase (POLG) locus associated with male infertility. Nat Genet **29**:261–262.

Ruhayel, Y., Lundin, K., Giwercman, Y., Hallden, C., Willen, M. and Giwercman, A. (2004) Androgen receptor gene GGN and CAG polymorphisms among severely oligozoospermic and azoospermic Swedish men. Hum Reprod **19**:2076–2083.

Sassone-Corsi, P. (1995) Transcription factor responsive to cAMP. Annu Rev Cell Dev Biol **11**:355–377.

Sharpe, R. M. and Skakkebaek, N. E. (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet **341**:1392–1395.

Singh, K., Singh, S. K., Sah, R., Singh, I. and Raman, R. (2005) Mutation C677T in the methylenetetrahydrofolate reductase gene is associated with male infertility in an Indian population. Int J Androl **28**:115–119.

Stouffs, K., Lissens, W., Tournaye, H., Van Steirteghem, A. and Liebaers, I. (2005) Possible role of USP26 in patients with severely impaired spermatogenesis. Eur J Hum Genet **13**:336–340.

Stuppia, L., Gatta, V., Scarciolla, O., Colosimo, A., Guanciali-Franchi, P., Calabrese, G. and Palka, G. (2003) The methylenetethrahydrofolate reductase (MTHFR) C677T polymorphism and male infertility in Italy. J Endocrinol Invest **26**:620–622.

Suzuki, Y., Sasagawa, I., Itoh, K., Ashida, J., Muroya, K. and Ogata, T. (2002) Estrogen receptor alpha gene polymorphism is associated with idiopathic azoospermia. Fertil Steril **78**:1341–1343.

Tanaka, H., Miyagawa, Y., Tsujimura, A., Matsumiya, K., Okuyama, A. and Nishimune, Y. (2003) Single nucleotide polymorphisms in the protamine-1 and -2 genes of fertile and infertile human male populations. Mol Hum Reprod **9**:69–73.

Teng, Y. N., Lin, Y. M., Lin, Y. H., Tsao, S. Y., Hsu, C. C., Lin, S. J., Tsai, W. C. and Kuo, P. L. (2002) Association of a single-nucleotide polymorphism of the deleted-in-azoospermia-like gene with susceptibility to spermatogenic failure. J Clin Endocrinol Metab **87**:5258–5264.

Tschanter, P., Kostova, E., Luetjens, C. M., Cooper, T. G., Nieschlag, E. and Gromoll, J. (2004) No association of the A260G and A386G DAZL single nucleotide polymorphisms with male infertility in a Caucasian population. Hum Reprod **19**:2771–2776.

Tut, T. G., Ghadessy, F. J., Trifiro, M. A., Pinsky, L. and Yong, E. L. (1997) Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab **82**:3777–3782.

Vogt, P. H. (2004) Genomic heterogeneity and instability of the AZF locus on the human Y chromosome. Mol Cell Endocrinol **224**:1–9.

Vouk, K., Hudler, P., Strmsnik, L., Fink, M., Majdic, G., Zorn, B., Lalli, E., Sassone-Corsi, P., Debeljak, N., Komel, R. and Rozman, D. (2005) Combinations of genetic changes in the human cAMP-responsive element modulator gene: A clue towards understanding some forms of male infertility? Mol Hum Reprod **11**:567–574.

Yang, X. J., Shinka, T., Nozawa, S., Yan, H. T., Yoshiike, M., Umeno, M., Sato, Y., Chen, G., Iwamoto, T. and Nakahori, Y. (2005) Survey of the two polymorphisms in DAZL, an autosomal candidate for the azoospermic factor, in Japanese infertile men and implications for male infertility. Mol Hum Reprod **11**:513–515.

Yen, P. H., Chai, N. N. and Salido, E. C. (1996) The human autosomal gene DAZLA: testis specificity and a candidate for male infertility. Hum Mol Genet **5**:2013–2017.

Yen, P. H. (2004) Putative biological functions of the DAZ family. Int J Androl **27**:125–129.

Yong, E. L., Loy, C. J. and Sim, K. S. (2003) Androgen receptor gene and male infertility. Hum Reprod Update **9**:1–7.

Yoshida, R., Fukami, M., Sasagawa, I., Hasegawa, T., Kamatani, N. and Ogata, T. (2005) Association of cryptorchidism with a specific haplotype of the estrogen receptor alpha gene: implication for the susceptibility to estrogenic environmental endocrine disruptors. J Clin Endocrinol Metab **90**:4716–4721.

Zhang, A. Z., Yang, S., Ma, Y., Lin, L. and Zhang, W. (2006) Single nucleotide polymorphisms of the gonadotrophin-regulated testicular helicase (GRTH) gene may be associated with the human spermatogenesis impairment. Hum Reprod **21**:755–759.

Zhoucun, A., Zhang, S., Yang, Y., Ma, Y., Zhang, W. and Lin, L. (2006) The common variant N372H in BRCA2 gene may be associated with idiopathic male infertility with azoospermia or severe oligozoospermia. Eur J Obstet Gynecol Reprod Biol **124**:61–64.

Zitzmann, M., Depenbusch, M., Gromoll, J. and Nieschlag, E. (2004) X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab **89**:6208–6217.