Spermatogenic failure. A genetic Odyssey
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CHAPTER 4

Idiopathic impaired spermatogenesis: genetic epidemiology is unlikely to provide a short cut to better understanding

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Abstract

The aetiology of impaired spermatogenesis is unknown in the majority of subfertile men. From several studies of concordance for involuntary childlessness among men we can conclude that there is a substantial familial component in male subfertility and shared loci segregating through families can be assumed. We now know that deletions on the Y chromosome, which do not penetrate fully, account for some of these cases. There are good reasons to suspect that other cases result from mutations in genes located elsewhere in the genome. In this article, we discuss different approaches to unravelling the molecular basis of impaired spermatogenesis originating from genetic abnormalities in chromosomes other than the Y chromosome.

Genetic mapping studies are in general a good approach to detect disease-causing genes that are segregating through a population; they can provide a shortcut to unravelling the biochemistry of a disease. In this paper, we explain our reasons for arguing that linkage and association studies are no promising means to identify the genes causing impaired spermatogenesis. We conclude that, direct screening of candidate genes for mutations will be necessary to detect genes involved in impaired spermatogenesis. However, this approach requires clues into the biochemical pathways of normal and abnormal spermatogenesis. Since we have a poor understanding of these pathways, more research is needed into the biochemistry of spermatogenesis.
Introduction

Subfertility, defined as one year of unprotected intercourse without conception, affects 10-15% of couples (Hull et al., 1985; De Kretser, 1997; Snick et al., 1997; Evers, 2002). Obviously, these figures depend on the definition of subfertility and the study population (Irvine, 1998). According to the World Health Organisation (WHO) in 47% of subfertile couples semen parameters are decreased (WHO 1987). The prevalence of subfertility exclusively based on testicular failure is not exactly known.

Evidently, subfertility is only diagnosed in couples that are trying to conceive a pregnancy. A combination of various female and male factors influences the fertility of a couple. Male subfertility can be categorized as due to pre-testicular, testicular and post-testicular factors (De Kretser, 1997). Endocrine disorders and sexual dysfunction are among the pre-testicular factors and epididymal or vasal obstruction is among the post-testicular factors. Testicular dysfunction results in reduced semen parameters due to impaired spermatogenesis. In this paper, we are interested in this group of otherwise healthy subfertile men with impaired spermatogenesis.

Several genetic abnormalities are involved in impaired spermatogenesis. Structural and numerical chromosomal abnormalities are found in approximately 4% of patients with azoo- or oligozoospermia (Tuering et al., 1998). Structural chromosomal aberrations cause meiotic abnormalities, resulting in spermatogenic failure (Chandley, 1979; Quack et al., 1988).

Five classes of Y chromosome deletions, AZFa (Azoospermia Factor a), P5/proximal-P1, P5/distal-P1, P4/distal-P1, and b2/b4 (AZFc) deletions, cause spermatogenic failure (Reijo et al., 1995; Vogt et al., 1996; Repping et al., 2002 and 2003). Deletions of the AZFc region are the most frequent and are found in 6-12% of azozoospermic or severely oligozoospermic men (Kremer et al., 1997; Kuroda-Kawaguchi et al., 2001).

In addition, mutations or an expanded CAG repeat length in the Androgen receptor (AR) gene have been described in subfertile men but strong arguments for a causal role are still lacking (Yong et al., 1998; Komori et al., 1999; Dowsing et al., 1999; Mifsud et al., 2001; Wallerand et al., 2001).

Idiopathic impaired spermatogenesis

In the majority of subfertile men, the aetiology of the impaired spermatogenesis is unknown (WHO 1987; Bhasin, et al., 1994; De Kretser, 1997). For clinical practice, this is not of great concern since treatment is not influenced by the cause of subfertility given the limited range of effective therapeutic options. Intrauterine insemination (IUI) is best first line treatment for moderate reduced semen parameters (Ombelet et al., 2003) and in vitro fertilisation (IVF) combined with intracytoplasmic sperm injection (ICSI) is the treatment of choice for
severe oligoasthenoteratozoospermia (Palermo et al., 1992; Tournaye 2000; Campbell and Irvine 2000). Surgically retrieved sperm cells from the epididymis or the testis can be used for ICSI in men with azoospermia (Palermo et al., 1999; Tournaye, 1999).

The therapeutic efficacy of ICSI is generally accepted but there is still concern about potential transmission of defects to the offspring (Bowen et al., 1998; Meschede et al., 1998; Johnson, 1998; te Velde et al., 1998; Campbell and Irvine 2000). Several follow up studies have been published in the past years. In most of these reports, no increase in the incidence of major birth defects has been described after ICSI (Palermo et al., 1996; Wennerholm et al., 2000; Sutcliffe et al., 2001; Bonduelle et al., 2002 and 2003). Only one follow up study reported a twofold higher risk of a major birth defect in ICSI children as in naturally conceived infants (Hansen et al., 2002). However, a recent follow up study of prenatal testing in 1586 ICSI conceived pregnancies indicated a higher risk for de novo chromosomal anomalies mainly related to a higher level of sex-chromosomal anomalies and also partly related to a higher level of de novo structural abnormalities (Bonduelle et al., 2002). The higher rate of aneuploidy in this series is most likely related to the higher aneuploidy rate in the sperm of the fathers.

Recently, some reports mentioned a higher incidence of Beckwith-Wiedemann syndrome and Angelman syndrome in children conceived by ICSI (Cox et al., 2002; DeBaun et al., 2003; Maher et al., 2003; Orstavik et al., 2003). In these syndromes imprinting defects play a causal role. Imprinting is a mechanism in which gene expression depends on the parental origin of the allele. It is hypothesized that the use of ICSI itself could increase the risk for imprinting disorders considering that the mammalian embryo, cultured in vitro, is susceptible to changes in imprinting control (Maher et al., 2003; Devroey and Van Steirteghem 2004). Until now, the evidence for the association between ICSI and imprinting disorders is based on case reports and uncontrolled cohorts. Therefore the exact impact of the risk is not yet clear and prospective controlled studies are needed to clarify whether imprinting disorders play a role in ICSI more often than in the general population (Devroey and Van Steirteghem, 2004; Gosden et al., 2003).

Y chromosome deletions however, are transmitted to sons by ICSI and therefore these boys are likely to be infertile as adults (Kamischke et al., 1999; Page et al., 1999). Although the fertility status of ICSI children is not known, as the oldest children are yet to enter puberty (Van Steirteghem et al., 2002), it is to be expected that if idiopathic impaired spermatogenesis has a genetic component, unknown genetic abnormalities are also transmitted via ICSI with a possible negative impact on fertility.

For this reason it is important to evaluate whether idiopathic impaired spermatogenesis does have a genetic component and whether it is a heritable condition. Furthermore, it is important to detect genes involved in testicular failure in order to implement diagnostic tools and offer appropriate counseling to patients who need ICSI. In this paper, we describe
what is known about the genetic background of idiopathic impaired spermatogenesis and discuss different approaches to identify the genes that may be involved.

**Genetic basis of idiopathic impaired spermatogenesis**

Genetic studies in several different animal models like the yeast *S. Cerevisiae*, the worm *C. Elegans*, the fly *Drosophila* and the mouse have provided evidence for the existence of hundreds of X chromosomal and autosomal genes that can mutate to male sterile alleles (Hackstein et al., 2000). From these studies it can be estimated that over 4000 genes may be involved in human spermatogenesis (Venables and Cooke, 2000).

The classical method, in humans, to identify whether a condition has a genetic basis is twin studies. In the case of male subfertility, only one underpowered pilot twin study has been performed so far. Despite its small size, this study showed a clear familial component to normal human spermatogenesis (Handelsman, 1997).

In addition to this study however, several case reports with multiple affected family members have been published. (Chaganti et al., 1979; Leonard et al., 1979; Shabtai et al., 1980; Cantu et al., 1981; Rivera et al., 1984; Meschede et al., 1994; Chang et al., 1999; Saut et al., 2000; Rolf et al., 2002; Tuerlings et al., 2002; Gianotten et al., 2003). In two families an autosomal recessive mode of inheritance was suggested but no cause for the shared infertility could be identified (Chaganti et al., 1979; Cantu et al., 1981). In one family an autosomal dominant trait of male infertility with sex-limited expression was suggested by segregation analysis (Tuerlings et al., 2002). In four families, structural chromosomal abnormalities were found (Leonard et al., 1979; Shabtai et al., 1980; Rivera et al., 1984; Meschede et al., 1994), while in three families a Y chromosome deletion was transmitted from the father to his infertile sons (Chang et al., 1999; Saut et al., 2000; Rolf et al., 2002; Gianotten et al., 2003).

These reports indicate that the affected family members share a genetic trait that accounts for their male subfertility. In addition, familial clustering of male subfertility has been observed in a case control study (Lilford et al., 1994). In this study, conclusions were based on a significantly increased number of subfertile brothers of men with reduced sperm counts as compared to fertile controls. Recently, we partly confirmed these data in a case control study comparing familial occurrence of male subfertility between families of patients with azoospermia or severe oligozoospermia and families of patients with normozoospermia (Gianotten et al., 2004). This study indicated that male subfertility due to impaired spermatogenesis has a familial component, but in only about half of the subfertile population.

Considering all these data, we can conclude that there is a familial component in male subfertility. This suggests a shared genetic or a shared environmental aetiology. As it is very likely that idiopathic impaired spermatogenesis has a genetic background; shared genetic
loci segregating through these families can be assumed.

**Genetic mapping in impaired spermatogenesis**
The strategy to identify disease-causing genes depends on how much is known about the pathogenesis of the disease and on what resources are available (Strachan and Read, 1999). As the pathogenesis of impaired spermatogenesis and the biochemical functions of the genes involved are not known currently, functional cloning strategies can not be used to identify the genes involved in human testicular failure. Positional cloning strategies on the other hand, are independent of the gene product and might therefore be a good approach for identifying the genes in impaired spermatogenesis. Positional cloning is based on genetic mapping. The aim of genetic mapping studies is to discover how often two loci are separated by meiotic recombination. This can be done by linkage analysis and by association analysis (Strachan and Read, 1999).

**Linkage studies in impaired spermatogenesis**
A marker is linked with a disease if there is a non-random co-segregation between the marker and the phenotype. Linkage analysis can be performed on the whole genome (multipoint analysis) as well as on specific candidate loci. Classical linkage analysis is performed on data of families with multiple affected family members. Linkage analysis using shared segment methods is used in nuclear families. The analysis of linkage studies can be very difficult for several reasons, which we will discuss in perspective to the male subfertility phenotype.
First, a classical linkage study requires large families with multiple affected family members in which co-segregation of numerous genome-wide polymorphic markers can be studied. However, families with the disease under study, especially when subfertility is studied, are seldom large enough for results from one family alone to reach statistical significance. Therefore, it is necessary to combine data from several families assuming that the genetic disease locus involved is the same in those families.
Second, to detect genes which are causing impaired spermatogenesis we are only interested in the subfertile males with reduced semen parameters and not in men of subfertile couples with normal semen parameters. In pedigrees however, the exact cause of subfertility is not always known and therefore in family studies, men with subfertility due to impaired spermatogenesis can not easily be identified. In addition, men who conceived children spontaneously will be indicated as fertile, even though they may have reduced semen parameters.
Third, in families with subfertile males the degree of unknown non-paternity might be higher than in the general population. This will influence the analysis of the family data negatively. The fourth problem is that conventional linkage studies require a specification of the genetic
model of the disease. Unfortunately, we can only speculate about the genetic model involved in impaired spermatogenesis, as there is no clear pattern of segregation. Determining the genetic model behind impaired spermatogenesis is difficult, as the phenotype influences the mode of inheritance by itself. An *autosomal recessive* mode of inheritance was the best fitting model in the majority of cases in both case-control studies (Lilford et al., 1994; Gianotten et al., 2004). Indeed, inheritance of autosomal recessive single gene defects might be one explanation how male factor subfertility can be transmitted to the next generation as only homozygotes are affected and recessive males would not be subfertile, but pass the defect to their children. However, *autosomal dominant* defects of maternal alleles or *X-chromosomal* aberrations might run in families as well and can possibly be transmitted through the maternal line. In addition, defects in mitochondrial DNA might also be involved since *mitochondrial* DNA is passed on to offspring only by mothers. Lastly, the results of the case control and twin studies are also compatible with cases arising from mutations of variable penetrance on the Y chromosome.

To resolve the problems of shortage of large families and the unknown genetic model, shared segment methods can be used instead of classical linkage analysis. Shared segment methods are model free linkage studies that can be used within nuclear families. Such analyses are much less powerful than conventional linkage studies but do not require any assumption on the underlying genetic model and unaffected people can be ignored in the analysis.

The most important difficulty however, is that linkage analysis in nuclear families as well as conventional linkage studies, identifies shared loci responsible for the phenotype. As human sperm cells and seminal plasma contains hundreds of different proteins, it has been suggested that many genes can potentially be involved in testicular failure (Lilford et al., 1994; Hackstein et al., 2000). Spermatogenesis is a complex process and problems at several different stages of spermatogenesis can result in male infertility. Therefore, probably many subgroups of patients with a different pathophysiologic background and thus with different genetic aberrations contribute to the male subfertile phenotype (Cooke and Saunders, 2002). In this respect, it is likely that many different genes and their interaction predispose for reduced semen parameters. These different disease loci will not be identified by linkage analysis.

In summary, because of all reasons mentioned above it is at this moment not realistic to expect that linkage studies are powerful enough to detect disease-causing genes involved in impaired spermatogenesis.

*Association studies in impaired spermatogenesis*

Linkage is a relationship between loci, but association is a relationship between alleles. Allelic association means that across the whole population, people who have a certain allele
at one locus have a statistically more than random chance of having a particular allele at a second locus (Strachan and Read, 1999). Association studies are generally performed on candidate genes but also polymorphic markers distributed over the whole genome can be analysed. This method offers the opportunity that unrelated affected subjects can be studied but results have to be compared with control subjects with the same genetic background. The ideal population for association studies is genetically homogeneous, because all case control studies are prone to selection bias (Strachan and Read, 1999). Moreover, the patients have to be descended from a small number of original founders.

In general, the statistical power to detect a real association is limited by the heterogeneity of a population due to environmental and other genetic factors. Therefore, in heterogeneous populations such as subfertile patients, large sample sizes are needed to obtain sufficient statistical power to detect genetic risk factors. More homogeneous populations such as genetically isolated populations might be an alternative, because environmental variation might be lower and the genetic background is expected to be less complex due to founder effects (Heutink and Oostra, 2002). However, this approach would miss different alleles, which might be important in other or more diverse populations.

As the patients under study in an association analysis are not directly related to each other, the chance that the disease phenotype is caused by a shared locus is even more unlikely than in linkage studies. Furthermore, in association studies, a candidate locus has to be defined and except the Y chromosome, we do not know of any specific regions with a high probability to be involved in impaired spermatogenesis.

In summary, association studies in testicular dysfunction have only a low probability to detect a disease locus involved in the male subfertile phenotype.

**Alternative genetic models**

Although familial clustering of male subfertility due to impaired spermatogenesis has been convincingly demonstrated, subfertility appears not to be segregating in the majority of the cases. Therefore, we might be dealing with *de novo* gene defects in a substantial number of cases and environmental factors can also play a role. Moreover, as it is likely that many genes are involved in impaired spermatogenesis, we have to consider more complex genetic models that theoretically could play a role in the subfertile phenotype.

In genetically *complex* disorders, which are diseases in which a *multifactorial* model plays a role, various susceptibility genes, each one with only moderate impact, influence the phenotype and exogenic non-genetic factors have substantial effects on phenotype expression. The aetiology of these disorders is therefore polygenic and there might be a reduced penetrance of the disease causing genes on the phenotype. In the *multifactorial major gene* model only a few major susceptibility genes affect the risk for reduced spermatogenesis, but their expression depends on smaller effects of minor susceptibility
genes and exogenous factors.

In the genetic *heterogeneity model*, different genes influence phenotype expression in different patients. In this model the phenotype is not a distinct disorder but includes several different disorders of distinct aetiology. For impaired spermatogenesis all models mentioned could play a role, each in a subgroup of patients, the so called *Mixed model*.

In addition to deletions of the Y chromosome, de novo mutations and these various genetic models, the phenomenon of *genetic imprinting* could also be important in impaired spermatogenesis. Imprinting is a mechanism in which the allele from one parent is expressed and the allele from the other parent is silenced (Hall, 1997). Because of this kind of differential expression, the effects of certain genes depend on whether they are inherited through the mother or through the father. Genetic imprinting has been observed in a number of disorders dealing with growth, behavior and abnormal cell growth (Hall, 1997). Imprinted genes show an unusual mode of inheritance, since mutant genes have an effect on the phenotype only if they come from the parent from which they are not silenced. Moreover, in several recent studies, it has been shown that allele-specific expression is also relatively common among non-imprinted autosomal genes (Knight, 2004). If differential expression plays a role in spermatogenesis, this will make the understanding of the molecular background of male subfertility even more complicated.

In summary, although impaired spermatogenesis has a familial component, the mode of inheritance is not clear. In addition to simple Mendelian segregation patterns and Y chromosome deletions, de novo mutations, more complex genetic models, environmental factors, reduced penetrance and allele-specific expression might be involved.

**Screening of candidate genes for mutations**

As we are probably looking for many different genes, and male subfertility might be a complex disease, we need a more direct approach to detect those genes than genetic mapping studies. Such an approach is screening of candidate genes by looking for sequence variations in cases which are not present in controls. A candidate gene is a gene that is considered, for different reasons, as a possible locus for the disease phenotype. If mutations in the gene under study can be identified in affected patients and not in the control samples, the gene is likely to be a locus for the disease (Strachan and Read, 1999).

In spermatogenic failure, the list of suggested candidate genes is exhaustive in accordance with the complex pathophysiology of spermatogenesis (Hackstein et al., 2000; Venables and Cooke, 2000; Cooke and Saunders, 2002; Matzuk and Lamb, 2002). In humans however, only very few candidate genes have been investigated until now.

Most research has been done on the Y chromosome. The first report indicating that the Y chromosome is involved in human spermatogenesis showed that some men with azoospermia had a deletion of the entire long arm of the Y chromosome (Tiepolo and
Since then five classes of Y chromosome deletions are described which cause spermatogenic failure; AZFa (Azoospermia Factor a), P5/proximal-P1, P5/distal-P1, P4/distal-P1, and b2/b4 (AZFc) deletions, (Reijo et al., 1995; Vogt et al., 1996; Repping et al., 2002 and 2003). Recently, the sequence of the male specific region of the human Y chromosome has been completed (Skaletsky et al., 2003). This region harbors at least 78 genes of which 60 are expressed exclusively or predominantly in the testis (Skaletsky et al., 2003). Until now, only one patient with a de novo point mutation in the USP9Y (Ubiquitin Specific Protease 9 Y) gene has been described resulting in azoospermia (Sun et al., 1999). Furthermore, several autosomal genes have been screened in DNA of subfertile men; the HOXA10 (Homeobox) gene (Kolon et al., 1999), the INSL3 (Leydig insuline-like hormone) gene (Krausz et al., 2000; Tomboc et al., 2000; Lim et al., 2001; Marin et al., 2001), the DAZL (Deleted in Azoosperma Like) gene (van Golde et al., 2001; Teng et al., 2002), the ZNF214 and ZNF215 (Zinc Finger) genes (Gianotten et al., 2003), the SYCP3 (Synaptonemal complex protein) gene (Miyamoto et al., 2003) and the HNRNP G-T (Heterogeneous nuclear ribonucleoprotein G-T) gene (Westerveld et al., 2004) respectively. Although several polymorphisms were identified in some of these genes, functional disruption of the genes could not be proven. The only exception is the SYCP3 gene, in which a deletion that results in a premature stop codon was identified in two of the 19 azoospermic patients with maturation arrest. This mutation is the only described autosomal gene defect that is proven causative in men with testicular failure.

Screening of candidate genes might be very appealing if the function of the candidate gene is known. To separate the biological relevant mutations from harmless SNPs (single nucleotide polymorphisms), the biological effect of each of the variants found in the mutation screen has to be tested (Heutink and Oostra, 2002). Unfortunately, in impaired spermatogenesis, the biological function of the genes is often unknown and therefore a causal role can only be assumed in case a mutation is identified. Another problem in screening candidate genes is that mutations can be located outside the coding region of the gene in elements that have a regulatory function of the gene (Heutink and Oostra, 2002). Such mutations will not be detected in screening the genes.

In summary, at this moment, mutation screening on candidate genes is the only realistic method to identify genes that might be involved in impaired spermatogenesis. However, candidate screening is time consuming, needs many well-defined patients and will reveal many negative results. This method would be more appealing if there is more knowledge about the biological function of candidate genes.
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Conclusions

Although there is a genetic basis for impaired spermatogenesis and in a subgroup of the population the phenotype segregates through their families, identifying the genes involved in impaired spermatogenesis is very difficult. It is very likely that male subfertility is a complex disease in which many susceptibility genes play a role. We discussed in this paper the reasons for arguing that linkage and association studies are no promising means to identify the genes causing testicular failure. At this moment, direct screening of candidate genes for mutations is the only realistic method to identify genes involved. However, this is a very time consuming method with a low probability to detect causal genes. To increase this probability, the number of good candidate genes has to be narrowed down. For example, this might be realized by expression studies. Moreover, more research is needed into the biochemistry of spermatogenesis in order to study the functional effect of genetic aberrations found by mutation screening in our patients.

References


Chapter 4


Ceneti cc epidemiology in idiopathic impaired spermatogenesis


Genetic epidemiology in idiopathic impaired spermatogenesis


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