Unravelling the genetics of spermatogenic failure

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Abstract

Subfertility, defined as the inability to conceive within 1 year of unprotected intercourse, affects 10–15% of couples. In up to 55% of couples, the male partner is diagnosed with spermatogenic failure, i.e. one or more semen parameters fall below the WHO criteria for normozoospermia. In these cases, assisted reproductive technology is usually used to achieve pregnancy. Both genetic and environmental factors are thought to underlie spermatogenic failure. Despite years of research, only few genetic factors have clearly been shown to cause spermatogenic failure, and the identification of additional genetic causes or risk factors has proven to be extremely difficult. In this review, we will present an overview of established genetic causes of spermatogenic failure, describe pitfalls in searching for novel genetic factors and discuss research opportunities for the future.


Male infertility: introduction to the problem

Subfertility is of major clinical, social and economical concern. In the western world, one in eight couples does not conceive spontaneously within 1 year of unprotected intercourse (de Kretser 1997). In up to 55% of couples seeking medical attention, the male partner is diagnosed with spermatogenic failure, defined as one or more semen parameters falling below the WHO cut-off for normozoospermia (De Kretser & Baker 1999). The prognostic value of this diagnosis is limited, as female parameters also contribute to the couple’s (sub)fertility. As semen parameters therefore cannot fully discriminate between fertile and subfertile men, reduced semen parameters are generally interpreted as a reduced chance of spontaneous pregnancy (Bonde et al. 1998, Guzick et al. 2001, Aitken 2006).

Less than 20 years ago, couples of which the man had azoospermia or severe oligozoospermia were doomed to remain childless. It was the introduction of ICSI that allowed these men to obtain offspring (Palermo et al. 1992). Subsequently, this technique was used successfully in men with ejaculatory azoospermia by injecting spermatozoa that were surgically retrieved from the epididymis (through microsurgical sperm aspiration) or testis (by testicular sperm extraction). At the same time, the development of ICSI spurred research into the genetic causes of low semen quality, following questions on the health status of children conceived using ICSI and the risk of transmitting spermatogenic failure to male ICSI offspring (Silber & Repping 2002). Follow-up data of ICSI children at 5 years of age indicate that, compared with naturally conceived children, children conceived through ICSI have an increased risk of congenital malformations, especially urogenital malformations in ICSI boys (Bonduelle et al. 2005).

Besides uncovering genetic causes of spermatogenic failure in light of diagnostic testing and counselling, the ultimate goal of studying spermatogenic failure is to develop a treatment for spermatogenic failure. Currently, the ‘treatment’ of spermatogenic failure consists of ovarian hyperstimulation of the unaffected female partner, which is both costly and burdensome and with potential unknown long-term side effects. Strikingly, as of today, there are no means to treat spermatogenic failure directly, except for treatment with a dopamine agonist in case of hyperprolactinaemia and treatment with gonadotrophins in case of hypogonadotrophic hypogonadism.

Known causes of spermatogenic failure

Spermatogenesis is governed by the parallel and serial actions of thousands of genes (Sha et al. 2002, Schultz et al. 2003, Schlecht et al. 2004, Ellis et al. 2007, Zamudio et al. 2008). In theory, alterations in any of these genes or their expression may cause spermatogenic failure. In reality, only a handful of genetic alterations have clearly been shown to cause spermatogenic failure (Nuti & Krausz 2008).

First, numerical and structural chromosomal abnormalities interfere with normal meiosis and can therefore cause spermatogenic failure (Tuerlings et al. 1998).
Klinefelter’s syndrome (47, XXY) is the most commonly found numerical abnormality. Structural abnormalities that are often encountered include reciprocal translocations, Robertsonian translocations and pericentric inversions (Tuerlings et al. 1998, Dohle et al. 2002).

Second, five recurrent Y-chromosome deletions, the so-called AZFa, P5/proximal P1 (AZFb), P5/distal P1, b2/b4 (AZFc) and gr/gr deletions, are associated with spermatogenic failure (Reijo et al. 1995, Vogt et al. 1996, Repping et al. 2002, 2003, Visser et al. 2009). These deletions all remove multiple genes that are potentially involved in normal human spermatogenesis. It is currently unknown which of these individual genes or any combination thereof play an essential role, and in which dosage (Noordam & Repping 2006).

Third, a number of monogenic disorders, listed in the Online Mendelian Inheritance in Man database, are associated with spermatogenic failure. Among these are Kallmann syndrome and Noonan syndrome. In monogenic disorders, spermatogenic failure results from hypogonadotropic hypogonadism, cryptorchidism, delayed puberty or male pseudohermaphroditism and ambiguous genitalia (Hardelin & Dode 2008). In cystic fibrosis (CF), spermatogenesis is unaffected, but congenital bilateral aplasia of the vas deferens (CBAVD) causes ejaculatory azoospermia. Besides being part of the disorder CF, CBAVD may also present without the other clinical symptoms common to CF, depending on the type of mutation in the CF transmembrane receptor gene. Although monogenic disorders are rare, they should not be overlooked in the workup of the male partner of the subfertile couple (Meschede & Horst 1997).

Apart from these established causes, there is a large body of literature either claiming or refuting the association of certain genetic variants with spermatogenic failure (Matzuk & Lamb 2008). Although the evidence in some of these studies is stronger than in others, the vast majority are single studies and for most of them no subsequent studies by other groups have confirmed the initial findings. Moreover, methodological and biological issues often confound these studies as outlined below.

Methodological issues in the study of the genetics of spermatogenic failure

First, many previous studies have included men based on their (reported) fertility, instead of their semen quality. Case–control association studies frequently included ‘subfertile’ men as cases and/or ‘fertile’, ‘proven fathers’ as controls without knowledge of their semen parameters. Since normozoospermic subfertile cases and oligozoospermic fertile controls are likely to have been included in these studies, no conclusions can be drawn with regard to the association between the genetic variant studied and spermatogenesis. It cannot be stated too often that subfertile is not the same as oligozoospermic, just as fertile is not synonymous with normozoospermic.

Second, due to the substantial interindividual variability of sperm counts, men may erroneously have been classified as normo- or oligozoospermic when only a single semen sample was used for classification (Tielemans et al. 1997, Alvarez et al. 2003).

Third, the case–control design in itself may not be the most suited design to study the effect of a putative genetic risk factor on spermatogenesis. Spermatogenesis is a quantitative, continuously distributed trait that shows large intra- and interindividual variation (Table 1). Therefore, a design that dichotomizes sperm counts is unable to detect a leftward shift of the sperm distribution curve, i.e. an overall reduction in sperm count, due to a genetic risk factor (Fig. 1).

Fourth, in many previous case–control studies, cases and controls were unmatched. Drawing cases and controls from different populations, for example using cases drawn from a clinical subfertile population and population samples as controls, may introduce selection bias. Another potential source of selection bias is the different ethnic background of included subjects. How to deal with these ‘racial’ differences is frequently debated in clinical, epidemiological and molecular research (Ioannidis et al. 2004). Some researchers argue that classification of patients according to ethnicity should be abandoned, because there would not be a correlation between common complex diseases and race, and genetic variation would be continuous and discordant with race (Cooper et al. 2003). However, there are known racial and ethnic differences in the cause, expression and prevalence of various diseases. Even with similar allele frequencies, i.e. similar genetic variation, the effect of a genetic risk factor may differ between ethnic groups (Burchard et al. 2003). In studies on spermatogenesis, geographic and ethnic differences in sperm quality have been reported between men in the Nordic–Baltic area (Jorgensen et al. 2002, Tserov et al. 2005). In addition, a possibly differential effect of POLG genotype on spermatogenesis was described in men from Dutch Caucasian or African descent (Westerveld et al. 2008a).

Table 1 Semen parameters in a cohort of 1014 consecutively included men, part of a subfertile couple, compared to WHO criteria.

<table>
<thead>
<tr>
<th>Semen parameter</th>
<th>WHO cut-off</th>
<th>Cohorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>2</td>
<td>3.3 ± 1.5</td>
</tr>
<tr>
<td>Concentration (10⁶/ml)</td>
<td>20</td>
<td>52 (16–89)</td>
</tr>
<tr>
<td>Motility (% progressive)</td>
<td>25</td>
<td>32 ± 16</td>
</tr>
<tr>
<td>Morphology (% normal)b</td>
<td>30</td>
<td>37 (25–46)</td>
</tr>
<tr>
<td>Total count (10⁶)</td>
<td>40</td>
<td>149 (44–281)</td>
</tr>
<tr>
<td>Total motile count (10⁶)</td>
<td>10</td>
<td>49 (7–110)</td>
</tr>
</tbody>
</table>

aData are presented as mean ± s.d. or median (25th–75th percentile).
bAccording to WHO (1992).
Large pedigrees are likely to be mutually exclusive, and linkage analysis is unlikely to provide novel findings. Segregation analysis has pointed to a heritable genetic factor underlying familial clustering of subfertility. Although the application of linkage analysis is preferable for genetic mapping, this method is limited to the next generation. This greatly hampers the chances of identifying a genetic locus strongly linked to spermatogenic failure, which is associated with limited reproductive success of this phenotype. If a genetic locus is identified, it is crucial to consider that both cases and controls are defined and selected on the basis of their respective semen parameters to avoid bias/confounding that goes with the use of subfertile cases and fertile controls.

Biological issues in the study of the genetics of spermatogenic failure

Besides the methodological issues described above, there are also some biological principles that should be taken into account when studying the genetics of spermatogenic failure. The first issue concerns the biological consequences of the spermatogenic failure phenotype, notably the limited reproductive success of this phenotype. If a genetic locus is strongly linked to spermatogenic failure, the chances are that this locus will not, or scarcely, be expressed in the next generation. This greatly hampers the application of linkage analysis, which is preferably carried out on large extended pedigrees. Although familial clustering of subfertility has been reported and segregation analysis has pointed to a heritable background, linkage analysis is unlikely to provide novel genetic loci, simply because spermatogenic failure and large pedigrees are likely to be mutually exclusive (Lilford et al. 1994, Gianotten et al. 2004).

Another biological issue relates to the characteristics of human spermatogenesis as compared with other mammals, notably rodents. Even though spermatogenesis in mice and men involves similar, highly evolutionary-conserved processes, there are also many different processes involved that are likely to be governed by different sets of genes. This may limit the usefulness of another method to search for genetic factors underlying spermatogenic failure, namely the mutation screening of candidate genes derived from mouse knockout models. To date, there are over 400 mouse models with a reproductive phenotype (Matzuk & Lamb 2008). Other studies providing candidate genes are those analysing expression profiles of rodent gametes throughout gametogenesis (Schultz et al. 2003, Schlecht et al. 2004, Chalmel et al. 2007). Although the generation of a multitude of mouse knockout models is of great value in fundamental research on mammalian fertility and spermatogenesis, the equivalents of artificially induced homozygous knockouts in mouse are indeed not commonly found in humans. In humans, it is usually heterozygous single base pair mutations or small deletions that are detected (Westerveld et al. 2005, 2006). Interestingly, in mouse knockout studies, the heterozygous deletion most often does not have a phenotype (O’Bryan & de Kretser 2006). The natural occurrence of heterozygous gene mutations rather than the artificially induced homozygous gene deletions in humans reflects the fact that thousands of genes are involved in spermatogenesis and hence each single mutant is likely to occur spontaneously at extremely low frequency. Another difference between mice and men is that, in contrast to inbred mouse models, non-inbred human populations harbour large variation in environmental and genetic factors that may modulate the effects of genetic variants.

Finally, an enduring complicating factor of studies in the field is the lack of functional assays to assess the biological effect of genetic variants found in men with spermatogenic failure. Such assays are unavailable, either because the function of the encoded protein is unknown, or because there is no suitable cell system to test its function. To date, no human spermatogonial stem cell (SSC) line is available to let alone an in vitro system for SSC differentiation. Although such systems are becoming available in rat and mouse, these might not be ideal to study human spermatogenesis as mentioned earlier (van Pelt et al. 2002, Kanatsu-Shinohara et al. 2003).

Investigating the genetic basis for low semen quality: the way forward

Evidently, all issues discussed above, both methodological as well as biological, should be taken into account when studying the genetic causes of spermatogenic failure. Below we will indicate how to deal with some of these issues.

When using a case–control design, it is a prerequisite that both cases and controls are defined and selected on the basis of their respective semen parameters to avoid bias/confounding that goes with the use of subfertile cases and fertile controls.

As it both avoids the introduction of a possible selection bias and does not require dichotomization of quantitative data, a cohort design is the most powerful to study genetic risk factors. By assembling a large cohort of men with varying sperm counts and comparing the sperm counts of men with and without a genetic variant, the effect of a genetic variant on the distribution of sperm counts of men with and without a genetic variant, the effect of a genetic variant on the distribution of sperm counts can be studied.
counts can be observed. Using this design, we were able to clearly refute an association between the CAG repeat in the androgen receptor and the CAG repeat in the polymerase γ-gene and spermatogenic failure (Westerveld et al. 2008a, 2008b).

Another way to improve the chances of identifying genes associated with spermatogenic failure is by selecting groups of men with a very homogeneous phenotype, for example isolated asthenozoospermia, isolated teratozoospermia, meiotic arrest or Sertoli cell-only syndrome. It may well be that the phenotypes that are usually studied (azoospermia and oligozoospermia) are too heterogeneous, and actually include men with different underlying (testicular) phenotypes. The advantage of this approach is illustrated by the fact that causal mutations in the SYCP3 and SPATA16 genes were found in groups of men with a very homogeneous phenotype, although for SYCP3 subsequent studies failed to find mutations in this gene (Miyamoto et al. 2003, Stouffs et al. 2005, Dam et al. 2007, Martinez et al. 2007).

Apart from the different methodological problems in previous studies, one technical issue applies to all of them. Nearly, all studies studied the effect of a single gene on spermatogenesis. As stated earlier, with thousands of genes involved in spermatogenesis and thus theoretically just as many in spermatogenic failure, chances of identifying individuals carrying one particular causal variant are extremely low. With the advent of high throughput screening methods, thousands of genes can now be studied at the same time. This should open up new opportunities in studying the genetic causes of spermatogenic failure (Aston & Carrell 2009). Besides investigating base pair mutations, attention should also be given to the so-called copy number variants (CNV), DNA segments of 1 kb or larger which are present at variable copy numbers in comparison with a reference genome, including deletions, duplications and insertions (Iafrate et al. 2004, Sebat et al. 2004, Feuk et al. 2006). CNVs make up a very large source of structural variation in the human genome and are thought to even outweigh the variation that is contained by single nucleotide polymorphisms (SNPs; Redon et al. 2006). Also, whole-genome expression arrays can be used to identify differentially transcribed genes in patients and controls. However, expression array data have to be interpreted with caution since differential expression can simply mirror the presence or absence of specific cell types in the testis rather than provide clues on which genes are involved.

The future of genetic studies in spermatogenic failure

Considering the few genetic factors that have so far been found to cause spermatogenic failure and the exhaustive lists of candidate genes, much work remains to be done. As outlined above, new insights should become available by 1) assembling large cohorts of men with 2) homogenous spermatogenesis-based phenotypes for 3) whole-genome analysis, at the level of SNPs, CNVs and eventually whole-genome sequencing. In addition, much progress can be made by establishing a human SSC culture and in vitro differentiation system to study the effects of genetic variants on human spermatogenesis. Hopefully, the findings from these types of studies will eventually lead to treatments for male subfertility.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the work/research reported.

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