Genetic basis of male fertility

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We are in the age of genetic discovery. Now the human genome has been completely sequenced, there will be increasing understanding and ability to manipulate biochemical pathways downstream of genes. At the same time, further development of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) will enable procreation in situations that were formerly impossible and when there may be an increased possibility of genetic abnormality. Furthermore, pre-implantation diagnosis will enable defects to be diagnosed and will give the opportunity for the couple to decide whether to continue with treatment towards a pregnancy or not. Thus, there is a need for clinicians to have a good knowledge of the genetic and hereditary aspects of male (and indeed female) infertility and for couples to have access to correct information and expert counselling. Also, there are ethical implications of these scientific and clinical advances for the future child, the individual, the couple and society. There is increasing public unease about this new science of reproduction and, in the UK, there is regulation by law; thus, there is a need for clinicians and scientists to give accurate information in everyday language to the public.

The normal human cell has 46 chromosomes arranged as 22 pairs of autosomes and either two X chromosomes in the female or an X and Y in the male. On these chromosomes are arranged approximately 50,000–100,000 genes that encode proteins. The chromosomes package contains 6–7 billion base pairs of deoxyribonucleic acid (DNA). The DNA is arranged as sequences of the four DNA bases (adenosine, cytosine, thiamine and guanine; ACTG) and it is this sequence that spells out the sequence of amino acids in proteins. Not all of the genetic code sequence translates into protein production. The sequences of the DNA bases are arranged into protein describing areas called exons, non-protein describing areas called introns and also special sequences to indicate the beginning and end of genes. Usually the sequence of amino acids in a protein is derived from information from several exons and processing is under the control of splicing enzymes. Splicing allows the formation of different proteins with shared common sequences to use the same exon and thus maximizes the efficiency of the genetic information.

Genes can be classified into functional classes; for example; oncogenes, check point genes, genes regulating apoptosis, etc. Genes may be arranged in
functional clusters and with respect to the male gender the sex-determining gene SRY is located on the short arm of the Y chromosome and there are several genes involved with spermatogenesis on the long arm of the Y chromosome.

Humans share common genes with all living creatures. We differ from our closest relatives the chimpanzees by approximately 1.6% of our DNA and diverged from a common ancestor about 7 million years ago. Gorillas differ in approximately 2.3% of their DNA from chimpanzees and man and diverged from our (humans and chimpanzees) common ancestor about 10 million years ago. All the above species diverged from monkeys about 32 million years ago and there is a DNA difference of only 7.5%\(^3\). Our kinship with primates and mammals allows appropriate animal models to be used to study human genetic mechanisms both generally and with respect to male fertility.

Spermatogenesis is unique because the DNA content of sperm is half that of the spermatogonia. In the initial stages, mitotic divisions give rise to spermatocytes. These undergo meiotic division to form haploid round spermatids. The round spermatids elongate in a process called spermiogenesis and, during this process, the DNA becomes compacted in the sperm head. These changes are under genetic control, but we are only now beginning to understand the mechanisms. It is estimated that there are 2000 genes that regulate spermatogenesis, most of these being present on the autosomes, but there are approximately 30 genes on the Y chromosome. Y genes are present in only half of humanity. In general, autosomal genes that regulate spermatogenesis are concerned with regulation of metabolic processes in other cells in the body as well as in the cells of spermatogenesis, whereas Y genes are not essential for vital functions outwith reproduction.

Y chromosome genes are subject to different evolutionary pressure compared with all other genes. Spermatogenesis is the result of rapid cell division and there is much greater opportunity for mutation than in oogenesis which occurs early in life and is followed by a long inert period. The Y chromosome has been present for all of evolutionary time in the mutagenic environment of spermatogenesis, whereas the X chromosome and all the autosomes have spent half of evolutionary time within the relatively inert environment of the ovum\(^4\). Normally, autosomal genes can undergo DNA repair when they pair with their opposite number during mitotic division, but for Y genes this pairing cannot happen. These differences in the environment of Y genes may explain the multicopy nature of some of the Y gene families and the many inert copies (pseudogenes) that are found. Unfortunately, this tendency for many copies makes it more difficult to map the Y chromosome accurately and more difficult to locate active genes. All Y genes are haploid and defects in a single gene are likely to have effects.

Our new understanding of the genetics of spermatogenesis holds the promise for the development of novel non-hormonal methods of male
contraception and this, in terms of overall scientific priorities for the human race, must rate very highly and is justification for funding this area of genetic research.

**Chromosomal abnormalities**

Disorders of the chromosomes can be classified as numerical or structural. Numerical disorders are polyploidy or aneuploidy. Polyploid cells (e.g. diploid [46 chromosomes], triploid [69 chromosomes], tetraploid [92 chromosomes]) are seen in spontaneous abortions and certain cancer cells and are essentially incompatible with life. Aneuploidy is where there is gain or loss of one or more chromosomes. In the context of male infertility, one of the most common aneuploid forms is 46XXY or Klinefelter's syndrome.

Structural chromosome disorders may be the deletion of material from one or more chromosomes, duplication of material, re-arrangement for the normal order within one chromosome or swapping of material between chromosomes; where there is no net loss of material this is known as a balanced translocation. A single chromosome may fuse with itself to form a ring. These structural abnormalities may lead to phenotypic disorder or may predispose to severe congenital abnormality when gametes are formed.

Chromosomal abnormalities are more common in infertile men and in subfertile male partners of ICSI couples. In a survey of pooled data from 11 publications reporting 9766 infertile men there was an incidence of chromosomal abnormalities of 5.8%. Of these, sex chromosome abnormalities accounted for 4.2% and autosomal abnormalities for 1.5%. For comparison, the incidence of abnormalities in pooled data from 3 series totalling 94,465 newborn male infants was 0.38% and of these 131 (0.14%) were sex chromosome abnormalities and 232 (0.25%) autosomal abnormalities. In a population of 781 male partners of couples undergoing ICSI, 30 men (3.8%) had chromosomal abnormalities; of these, there were 10 (1.2%) sex chromosome aberrations and 20 (2.6%) autosomal aberrations. There is less information about the incidence of chromosome abnormalities in spermatozoa. It is now possible to study large numbers of sperm using multicolour fluorescent in situ hybridization analysis (FISH), whereas previously sperm karyotyping was by hamster penetration assay – a method that was both laborious and insensitive. Two studies using FISH reveal increased frequency of aneuploidy in spermatozoa involving the autosomes and particularly the sex chromosomes. There is a need for further FISH sperm studies using sperm from men in well-defined clinical categories (e.g. Table 3). Also, when considering such studies, it may be relevant to study the population of sperm that would normally be used in an ICSI procedure. The ICSI
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biologist will normally select by motility and secondarily by morphology and it is probably appropriate to select in the same way sperm for chromosome studies.

Sex chromosomal abnormalities

Klinefelter's syndrome and variants (47XXY, 46XY;47XXY mosaicism)

Klinefelter's syndrome is the most frequent sex chromosome abnormality, which occurred in 66 (0.07%) of phenotypically newborn males in pooled data from cytogenetic analysis of 94,465 newborn infants. Adult males with Klinefelter's syndrome have small firm testicles that are devoid of germ cells; this is to be distinguished from men with other causes of damage to spermatogenesis who have small soft testicles. The phenotype can vary from a normally virile man to one with the stigmata of androgen deficiency including female hair distribution, scanty body hair, and long arms and legs, because of late epiphyseal closure.

Leydig cell function is commonly impaired in men with Klinefelter's syndrome. Testosterone levels may be normal or low, oestradiol levels normal or increased and FSH levels increased. Surprisingly, libido is often normal despite low testosterone levels, but with ageing there is often a need for androgen replacement; thus men with Klinefelter's syndrome should be offered longer-term supervision in addition to the management of their fertility problems.

Men with Klinefelter's mosaicism 46XY;47XXY have variable germ cell presence and variable sperm production. Until the advent of IVF and ICSI this was academic, but it is now important to diagnose mosaicism, as some sperm retrieved may be expected to be normal and can be used to fertilize. Pre-implantation FISH analysis of cells from embryos can be used to confirm normality. The production of 24XY sperm has been reported in 0.9-2.1% of men with Klinefelter's mosaicism and in 1.36-25% of men with somatic karyotype 47XXY. All this indicates that some 47XXY cells are able to achieve meiosis and produce mature spermatozoa. Conversely, it is not known whether haploid sperm in Klinefelter's syndrome are always the result of a clone of normal cells in a mosaic population or whether in certain circumstances some 47XXY male germ cells are viable and capable of producing haploid sperm.

Increased frequency of sex chromosome abnormalities in ICSI babies

There are a number of reports that indicate higher than normal frequency of sex chromosome abnormalities in children born of ICSI compared with...
the normal population. The underlying mechanism is not yet known, but there are several possibilities discussed in the literature: these include Klinefelter's mosaicism with an aneuploid cell line confined to the germ cells and thus not detectable by peripheral blood karyotyping, or the production of s47XY sperm chromosome diploid sperm and this has reported in sperm from severely oligozoospermic men with a normal karyotype. Another possibility is that low levels of mosaicism may be more common than is thought and may be missed during routine karyotype analysis.

47XYY a rarer abnormality and often associated with an apparently normal sperm analysis

Males with 47XYY are seen more frequently in the infertile population and FISH analysis of spermatozoa indicates an increase in disomy.

Y chromosome haplotype and male fertility

In 1992, there was a report of decreasing human sperm concentration over a number of years. This observation has been confirmed by others, although there is conflicting information from different countries. The most accepted hypothesis is that this is a deleterious effect of environmental contamination with oestrogenic chemicals. However, much less is known about the genetic contribution to male fertility potential. A Japanese group used PCR to look at DNA differences in the Y chromosome. The results were used to classify the Y chromosome into four haplotypes – I, II, III, IV. Men with haplotype II had a lower sperm concentration compared with men with haplotypes III and IV, and the frequency of haplotype II is more common in azoospermic men compared with normal men. This work indicates a significant genetic contribution to male fertility potential and could provide the basis for alternative hypotheses to explain changes in sperm concentrations in different populations with time.

Autosomal abnormalities

It is well known that apart from those men with Klinefelter's syndrome there is an excess of autosomal abnormalities in populations of men with non-obstructive azoospermia or severe oligozoospermia. Usually, autosomal disorders do not cause infertility in isolation, but reduced spermatogenesis is the consequence of a more general
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Disturbance in phenotype; patients with these problems are often known to doctors because of other developmental abnormalities and do not present de novo with infertility. However, in view of the known excess of these disorders in men with a low sperm count, it is appropriate to perform karyotyping for all men with low sperm counts where it is proposed to use ICSI to enable fertility and to offer genetic counselling when abnormalities are discovered. Karyotype analysis may be normal, but this does not exclude genetic defects; patients and infertility clinicians need to understand the limitations of karyotype analysis. From time to time, men may ask for ICSI where there is already a known autosomal defect. In these cases, the genetic counselling is the same normal and independent of the need for ICSI.

Genetic defects

Disorders of the sequence of the DNA bases are called mutations and, while mutations may occasionally be beneficial and become preserved in evolution, often they result in defective genetic function. It is also likely that many mutations occur in the testis during spermatogenesis and that the external position of the testes is because a cooler temperature damps down the mutation rate. Mutations may involve relatively large portions of the gene, but there are also many examples of where the alteration of a single base pair on the original DNA (point mutation) can have a profound effect on phenotype. Genetic defects may be single or multiple and may be inherited or the result of new mutation. They may be inherited in a dominant pattern, as in Huntington’s chorea, or a recessive pattern, as in cystic fibrosis, and may have variable penetrance.

When mutations are in exons, there is an alteration in the protein produced and, in many cases, a marked resultant alteration in phenotype. Mutations of non-coding areas (introns) are less well understood, but can affect the amount of protein produced making the synthesis of protein from the original DNA less efficient; for the andrologist, one of the best known examples of this is the 5T intron abnormality that occurs in the cystic fibrosis transmembrane gene complex (CFTR) and which is found either alone or with exon mutations in men with congenital absence of the vas deferens (see below).

Most, if not all, genes on the Y chromosome are to do with the regulation of male differentiation and defects will be manifest as phenotypic abnormality except where there are autosomal homologues of the gene, e.g. DAZ. X-linked genes may be dominant (e.g. hypophosphatemic rickets) or recessive (Duchene muscular dystrophy). X-linked dominant genes will alter the phenotype in the female, but in the male the recessive defect will also be manifest.
X linked genetic disorders and male fertility

Each man has only one X chromosome and, therefore, any X-linked recessive disorders will be manifest in males and the defect will be transmitted through his daughters to his grandsons.

Kallman's syndrome

The commonest X-linked disorder in infertility practice is Kallman's syndrome and the most common form of this is X-linked recessive caused by a mutation in the KALIG-1 gene on Xp22.3. This gene is involved with the regulation of cell adhesion and axonal path finding. Patients with Kallman's syndrome have hypogonadotrophic hypogonadism and may have other clinical features including anosmia, facial asymmetry, cleft palate, colour blindness, deafness, maldescended testes and renal abnormalities. Other rare forms of Kallman's syndrome have been described included an autosomal dominant form and autosomal recessive. It is important to note some men with Kallman's syndrome have an isolated gonadotrophin deficiency without any other phenotypic abnormalities and that these men may sometimes present de novo with infertility, which can be treated successfully by hormone replacement therapy.

Androgen insensitivity – Reifensteine syndrome

The rare disorder of androgen insensitivity may sometimes first present with infertility. The condition has X-linked recessive inheritance due to a defect in the androgen receptor gene located on Xq11-12. The phenotype may range from complete testicular feminisation with an immature female phenotype to an apparently normal male with infertility, although the latter is rare. In our clinic, we conducted a structured genetic search for androgen deficiency amongst those men with high testosterone and low sperm counts, but failed to find any cases using base pair mismatch analysis technology. In the course of the study, we reported several de novo mutations of the androgen receptor, but in all cases these were associated with obvious genital abnormalities, such as hypospadias. We concluded that androgen insensitivity in the infertile male in the absence of any genital abnormality is rare.

Other X disorders

There is a case report of an azoospermic man with biopsy proven spermatogenetic arrest who has been found to have a submicroscopic
interstitial deletion on the Xp pseudoautosomal region in peripheral blood and skin fibroblast samples. Other genetic and chromosome studies were entirely normal including probing of the Yq region. It is also worth noting the report of two men with azoosperma and X pseudoautosomal deletions. So far, there are no other examples of X-linked disorders affecting male fertility and, if such disorders exist and are not associated with azoosperma, these would skip generations of males and will be very difficult to define.

**X-linked disorders that are not associated with male infertility**

There are many rare X-linked disorders not associated with infertility. When recessive these appear in male babies but skip several generations and, therefore, family history is important. For example, Menkes disease is an X-linked recessive disturbance of copper metabolism associated with progressive neurological symptoms. The main problem is the recognition of such disorders especially if there has been several generations of female births where a recessive X-linked has been carried through these generations without any clinical stigmata. Management is in the normal way by giving the couple choices after appropriate genetic counselling, including consideration about the severity of any disorder that may result. It may be appropriate to offer the couple pre-implantation sex determination and replacement of female embryos only or, in some situations, not to embark on fertility treatment at all.

**Y genes and male infertility**

In 1992, we reported three men with severe damage to spermatogenesis and an apparently normal chromosome analysis, but where molecular probes revealed microdeletions on the long arm of the Y chromosome. We were stimulated to probe the long arm of the Y chromosome because of a report by Tiepolo and Zuffardi who described men with azoospermia and deletions of the long arm of the Y chromosome transecting interval 6 with the loss of all distal genetic material and by our own finding of an infertile man with a short arm dicentric Y. Following our 1992 report, there have been a large number of publications of case series and it is clear that, while microdeletions may occur in the fertile population, they are more prevalent in the infertile populations. To date, the microdeletions detected have been rather large, but there are preliminary reports of much smaller deletions within genes. Microdeletions have been found in three non-overlapping regions of the Y chromosome AZF a-b-c. Several genes have been described and these
Fig. 1 The Y chromosome and fertility genes. Figure prepared by Dr N Affara (Cambridge University) and presented at the Y Gene Conference, Royal College of Physicians, Edinburgh, September 1998.
include RBM\textsuperscript{39}, DAZ, DFFRY\textsuperscript{40}, DBY and CDY (Fig. 1). The abnormality most commonly reported in the literature is a microdeletion in the AZFc region and encompassing the DAZ gene. However, there is no exact correlation between DAZ deletion and the presence of absence of spermatogenesis, but this may be because for the DAZ gene there is also an autosomal copy (see below).

**DAZ and RBM genes**

The first candidate gene for spermatogenesis was identified from our unit and is now called RBM (RNA binding motif). There is a family of up to 50 RBM genes\textsuperscript{41} and, while most copies are probably inactive, deletions of the AZFb region causes functional inactivation of RBM. The DAZ gene (formerly known as SPGY) was the second candidate gene to be described; initially it was thought to be a single copy gene\textsuperscript{42}, but it is now known to be a member of a family of 6–10 genes\textsuperscript{44,45}. Both DAZ and the RBM gene families encode proteins with a similar structure, which are probably involved in the metabolism of RNA. RBM is a nuclear protein and its expression is restricted to the male germ line in humans and in mice, whereas DAZ protein is cytoplasmic. RBM and DAZ proteins are related to the protein hnRNPG and this family of proteins are involved in RNA metabolism including packaging of RNA, transport to the cytoplasm and splicing. However, hnRNPG is autosomally encoded and ubiquitously expressed indicating a function that is required for all cell types\textsuperscript{46–48}. RBM is probably regulating splicing events essential for spermatogenesis\textsuperscript{49}, whereas DAZ/SPGY with its autosomal contribution is important for gametogenesis in both sexes, perhaps in the male regulating translational repression during spermatogenesis; knockout female mice without the DAZL gene have a failure of proper development of the female genital tract.

RBM is a highly conserved gene and has been found on the Y chromosomes of all mammalian species so far tested\textsuperscript{50}, including marsupials\textsuperscript{51}. DAZ/SPGY is found only in humans and old world primates, but an autosomal homologue is present in mammals and in mice is found on chromosome 3. This autosomal homologue is also present in humans on chromosome 17 and may account for the varied spermatogenesis in men with AZFc deletions. DAZL protein is expressed only in male and female germ cells in mice and men and these observations have led to the suggestion that RBM and DAZLA might have been acquired from their respective autosomal homologue during the course of evolution and that genes associated with spermatogenesis will tend to accumulate on the Y chromosome. There is considerable sequence identity between the X and the Y and it has also been postulated that there has been inactivation of X genes leaving the active copy on the Y\textsuperscript{52,53}. 

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Other Y genes

Several other genes on the long arm of the Y have been described (Fig. 1), but less is known about the clinical importance of these as, in general, microdeletions in regions other than AZFc are less frequent. Also, the interaction between genes may be complex; for example, in the case of DFFRY and DBY genes, there is interaction between the two genes so that complete azoospermia only occurs when both genes are deleted together.

Clinical implications of Y microdeletions

There are no reports that men with microdeletions have any phenotypic abnormalities other than abnormal spermatogenesis and men with microdeletions appear to be in perfect health in every other respect. As there is only one Y chromosome, we may predict that Y microdeletions will be transmitted to their sons although this is likely to be rare in the normal population because without treatment with ICSI men with very low sperm counts are less likely to be father of children. However, four such cases have been reported in the literature (Table 1). It may be important that in two of the cases the microdeletion appears to be larger.

Table 1  Transmission of Y chromosome deletion from father to son

<table>
<thead>
<tr>
<th>Authors</th>
<th>Y deletion son</th>
<th>Y deletion father</th>
<th>Phenotype son</th>
</tr>
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<tbody>
<tr>
<td>Kobayashi et al 1994</td>
<td>AZFc</td>
<td>AZFc</td>
<td>7</td>
</tr>
<tr>
<td>Vogt et al 1996</td>
<td>AZFc</td>
<td>AZFc</td>
<td>&lt; 0.01 x 10^6</td>
</tr>
<tr>
<td>Kent-First et al 1996</td>
<td>Small near AZFc</td>
<td>Small near AZFc</td>
<td>ICSI</td>
</tr>
<tr>
<td></td>
<td>Small near AZFc</td>
<td>Not detected</td>
<td>Normal</td>
</tr>
<tr>
<td>Pryor et al 1997</td>
<td>SY153-SY267</td>
<td>SY153-SY267</td>
<td>0.3 x 10^6</td>
</tr>
<tr>
<td></td>
<td>SY207-SY272</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>Stuppia et al 1997</td>
<td>AZFc</td>
<td>AZFc (smaller)</td>
<td>&lt; 2 x 10^6</td>
</tr>
<tr>
<td>Mulhall et al 1997</td>
<td>Ongoing twin pregnancy</td>
<td>AZFc</td>
<td>ICSI</td>
</tr>
<tr>
<td>Silber et al 1998</td>
<td>Two ongoing pregnancies</td>
<td>AZFc Azoospermic</td>
<td>TESA-ICSI</td>
</tr>
<tr>
<td></td>
<td>Two ongoing, 1 twin AZFc</td>
<td>AZFc oligospermic</td>
<td>ICSI</td>
</tr>
<tr>
<td>Kamischke et al 1999</td>
<td>AZFc</td>
<td>AZFc</td>
<td>ICSI</td>
</tr>
</tbody>
</table>

Adapted from a table presented by Dr K McElreavey, Institute Pasteur, Paris at the Y Gene Conference, Royal College of Physicians Edinburgh, 1998
in the son than the father. More information is needed from father/son pairs where the son has a very low sperm count and also about the outcome of ICSI attempts where sperm have been used from men with microdeletions. There is a need for long-term follow-up of any male children. However, although it may be desirable to obtain information about the genetic status of ICSI babies, there are ethical questions about whether young babies should be tested and, if so, whether the test results should be identifiable.

**Testing for Y microdeletions**

Testing for microdeletions is now widespread in IVF/ICSI units, but there is no standardised methodology and, therefore, it is difficult to make direct comparison between reported results. Several centres have developed screening methodologies.[38,54-56]. As there is no correlation between histopathology and deletion of DAZ, it is premature to rely on specific gene probes as these will fail to detect a significant proportion of men with microdeletions. Also testing using peripheral blood may not be reliable. Lack of DAZ mRNA in testicular cells has been reported in a man with apparently normal DAZ gene constitution on DNA extraction from leukocytes.[57]. These findings may be explained by unrecognized very small deletions encompassing active copies of DAZ, mosaicism, or abnormalities of DAZ transcription.

**Advice to couples where the man has a Y microdeletion**

What advice can we give our patients? There is no point in testing men for microdeletions where ICSI is being used to overcome obstructive azoospermia, as in these men spermatogenesis should be normal. For other men with severely damaged spermatogenesis, testing for microdeletions before ICSI is desirable; but, as these men and their male children are unlikely to have any phenotypic abnormality other than damaged spermatogenesis, it is reasonable to take into account the cost and limitations of present methods of testing and to discuss this with the couple. If a man with microdeletions and his partner wish to proceed with ICSI, they can be advised that microdeletions will be passed to sons but not to daughters; however, it is unknown to what extent a son who inherits a microdeletion will, in turn, have a fertility problem. The couple may be told that there is no evidence of any other health consequences of microdeletions. In a study of the actual decisions taken by couples in this situation in The Netherlands and Belgium, it was found that most chose to proceed with ICSI but that 21% refrained from treatment or chose DI; the decision was strongly influenced by the opinion of the counsellor.[58].
Autosomal defects with severe phenotypic abnormalities as well as infertility

There are a number of inherited disorders with severe or considerable generalized abnormalities as well as infertility (Table 2). Such patients will be well-known to doctors often from childhood and any fertility problem has to be managed in the context of the care of the man as a whole and with consideration of his and his partner's ability to care for a child should treatment be successful.

Autosomal defects causing male infertility but in the absence of overt phenotypic abnormality

Lillford et al\(^9\) published an epidemiological study indicating that reduced male fertility may run in some families and there is some molecular evidence that there may be autosomal defects that could account for this. It is difficult to know how many autosomal genes are specific for fertility, but there are likely to be relatively few as with evolution these tend to accumulate on the Y chromosome.

Roest et al reported a mutation affecting the ubiquitin conjugating enzyme hHR6A and hHR6B in mice\(^60\). This enzyme is implicated in post-replication repair. Heterozygous male mice and knockout female mice are completely normal and able to transmit the defect which, however, caused deranged spermatogenesis in homozygous mice. It is possible that similar hHR6B mutations may cause male infertility in man. If this is the case, then there may be an enhanced chance of passing such defect by ICSI as the male partner will be more likely than normal to carry such a defect and will

Table 2 Less common inherited disorders associated with infertility and other alterations to phenotype

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Phenotype</th>
<th>Genetic basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prader-Willi</td>
<td>Obesity, mental retardation</td>
<td>Deletion of 15q12 on paternally inherited chromosome</td>
</tr>
<tr>
<td>Bardet-Biedle</td>
<td>Obesity, mental retardation, retinitis pigmentosa, polydactyly</td>
<td>Autosomal recessive 16q21</td>
</tr>
<tr>
<td>Cerebellar ataxia and hypogonadotrophic hypogonadism</td>
<td>Eunuchoidism and disturbances of gait and speech</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Noonan's syndrome</td>
<td>Short stature, webbed neck, cardiac and pulmonary abnormality, cryptorchidism</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
<td>Muscle wasting, cataract testicular atrophy</td>
<td>Autosomal dominant 19q13.3</td>
</tr>
<tr>
<td>Dominant polycystic kidney disease</td>
<td>Renal cysts, obstruction from epididymal cysts</td>
<td>Autosomal dominant 16p13 3 and 4q</td>
</tr>
<tr>
<td>5-α-reductase deficiency</td>
<td>Perineal or scrotal hypospadias, vaginal pouch immature female phenotype</td>
<td>Autosomal recessive</td>
</tr>
</tbody>
</table>
thus pass the defect to half of his offspring. The chance of the offspring being affected will depend on the prevalence rate of these mutations in the general community. This may be quite low as there will have been a tendency to select against such mutations because of their antifertility effect.

Cystic fibrosis mutations and male infertility

Cystic fibrosis, a fatal autosomal recessive disorder is the most common genetic disease of Caucasians; 1 in 25 are carriers of gene mutations involving the cystic fibrosis transmembrane conductance regulator gene (CFTR). This gene, located on the short arm of chromosome 7, encodes for a membrane protein that functions as an ion channel and also influences the formation of the ejaculatory duct, seminal vesicle, vas deferens and distal two-thirds of the epididymis. Congenital bilateral absence of the vas deferens is associated with CFTR mutations and is found in approximately 2% of men with obstructive azoospermia attending our clinic in Edinburgh. However, the incidence in men with obstructive azoospermia will vary in different countries depending on the prevalence of cystic fibrosis mutations in the population and the prevalence of other causes of obstruction; in those countries with a high prevalence of sexually transmitted infection, CBAVD as a cause of azoospermia will be relatively infrequent compared with azoospermia associated with post gonococcal epididymitis. However, the clinical finding of absent vasa is easy to miss and all men with azoospermia should be very carefully examined to exclude CBAVD and particularly if semen analysis reveals azoospermia in association with a semen volume of less than 1.0 ml and the pH is acidic (<7.0).

In recent years, increasing numbers of mutations of the CFTR gene have been characterized and more than 400 have been described. There have been at least 17 published series of men with CBAVD who have been tested for varying numbers of mutations. In general, the more mutations tested for, the higher the percentage of men found to have mutations, so that in more recent publications detection rates have been higher (75%, 70%, 81%, 76%, respectively) whereas in older publications detection rates have been around 40%. In a review of published series of 449 men with CBAVD, the Delta F508 mutation was detected in 244 men, the R117H mutation in 54 men and the W1282X mutation in 37; additionally, 63 other mutations were detected in between 1–9 men, but not all mutations were tested for in all case series. It seems likely that, as more and more mutations are defined and tested for, approaching 100% of men with CBAVD will be found to have mutations. At present it is not practical to test for all known mutations as many have a very low prevalence in a particular populations and in
most places testing is restricted to the 20–30 mutations that occur most commonly in that community.

Mutations may be found in both copies of the CFTR gene, but in most men with CBAVD they are found in only one copy. In some of these supposedly heterozygous cases there may be an unknown second mutation, but there is also another interesting mechanism. In up to 63% of these, a DNA variant the 5T allele can be detected in a non-coding region of CFTR and we have confirmed these observations in our own patients. Further work is needed to understand the genetics of CBAVD fully. It is noteworthy that the heterozygous men with CBAVD whom we see in our clinic often have mild clinical stigmata of cystic fibrosis, e.g. history of chest infections. It will, therefore, be important to follow-up children born after ICSI and where the father has CBAVD and is either hetero or homozygous. It is also worth advising those men with any mild clinical stigmata to avoid smoking as their respiratory reserve will be reduced compared with normal.

There have been reports of CFTR mutations in men with severe oligozoospermia, but without absence of the vas deferens, and it has been postulated that the CFTR complex may also relate to spermatogenesis. While the relationship between absence of the vas deferens and CFTR mutations is becoming well established, the role of these mutations in spermatogenetic defects is as yet unclear.

Advice for couples where the man has CBAVD

CFTR mutations have implications for clinical infertility practice. When the male partner has CBAVD, it is important to test the female partner for CF mutations as well. If she is also found to be a carrier, then there must be very careful consideration about whether the couple wishes to proceed with ICSI using the husband’s sperm as the chance of a baby with cystic fibrosis will be 25% if he is heterozygous or 50% if he is homozygous. If the female partner is negative for known mutations, her chance of being a carrier of unknown mutations is about 0.4% and, in these circumstances, the chance of her heterozygous partner siring a child with cystic fibrosis is approximately 1:410. These figures are estimates calculated in the light of known mutation frequency in Caucasian populations, but will vary depending on the frequency of the different mutations in different populations.

Unilateral (and bilateral absence/abnormality of the vas) and renal abnormalities – a different genetic disorder

Unilateral absence of the vas deferens is usually associated with ipsilateral absence of the kidney. This probably has a different genetic cause and is
most commonly encountered as an incidental finding in the vasectomy clinic. CF tests should be performed when a man is found to have a unilateral absence of the vas and the kidneys are normal; conversely, when a man has bilateral vas abnormality and CF tests are normal, then ultrasound is indicated to define renal anatomy.

**Mitochondrial abnormalities**

Very few, if any, mitochondria are a parental contribution to the embryo and, therefore, these are unlikely to be a significant factor in inherited male infertility, although clearly defective mitochondrial function in sperm may, by way of poor sperm motility, be a cause of infertility.

**Cytoplasmic disorders that may be inherited**

There is evidence that in humans the cytoskeleton is a paternal contribution and thus, in theory, there is the possibility of non-DNA transmitted abnormalities when very defective sperm is used. Whether this actually occurs is not known.

**Unknown genetic disorders**

Intracytoplasmic sperm injection (ICSI) is now used to enable men with severe damage to spermatogenesis to father children in situations formerly considered hopeless and where only a very few spermatozoa can be obtained. This has led to worries that children may be born with fetal abnormality because, by bypassing the selective processes of the female genital tract and coverings of the egg, the process could enable defective sperm to fertilize or, alternatively, eggs may be fertilized that would otherwise not do so. It is, therefore, very re-assuring that the collected statistics of fetal abnormality from ICSI centres do not indicate any increase in congenital malformations compared with the general population. However, the indications for ICSI are constantly being extended to include fertilisation with immature sperm forms and it will be particularly important to continue to monitor fetal abnormality rates with detailed subgroup analysis according to the clinical and molecular diagnosis of the father (see Table 3).

**Ethical considerations, genetic counselling and ICSI**

The main difficulties will occur where there is a conflict of interest between the wishes of the couple and the interests of a future child.
<table>
<thead>
<tr>
<th>Suggested male risk categories for the follow up of children born by ICSI</th>
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<tbody>
<tr>
<td><strong>1</strong> Men with sex chromosome abnormality on peripheral blood karyotyping</td>
</tr>
<tr>
<td><strong>2</strong> Men with autosomal chromosome abnormality on peripheral blood karyotyping</td>
</tr>
<tr>
<td><strong>3</strong> Men with obstructive azoospermia secondary to CBAVD. This category has been discussed above</td>
</tr>
<tr>
<td><strong>4</strong> Men with damaged spermatogenesis and Y microdeletions, again discussed above</td>
</tr>
<tr>
<td><strong>5</strong> Men with other defined genetic disorders. Some possibilities have been discussed above</td>
</tr>
<tr>
<td><strong>6</strong> Men with normal spermatogenesis and obstructive azoospermia, for example after failed vasectomy reversal but where karyotyping and genetic tests have not been undertaken or if they have been performed and are normal. In this category, there is no reason to predict any genetic abnormality different from the general population</td>
</tr>
<tr>
<td><strong>7</strong> Men with normal karyotype and normal genetic tests but where spermatids have been used. In cases where karyotype or genetic tests are abnormal, then the followed up category should probably be considered under one of the above groups</td>
</tr>
<tr>
<td><strong>8</strong> Men with testicular maldescent</td>
</tr>
<tr>
<td><strong>9</strong> Men with damaged spermatogenesis secondary to cancer chemotherapy</td>
</tr>
<tr>
<td><strong>10</strong> Men with damaged spermatogenesis secondary to known mterigen, e.g. occupation hazard</td>
</tr>
<tr>
<td><strong>11</strong> Men with damaged spermatogenesis of unknown aetiology</td>
</tr>
</tbody>
</table>

The male diagnostic categories descend in order of genetic and diagnostic precision. The actual risk to the potential child of fathers in these categories is unknown, but use of pooled category data will help define risks which will not be apparent from even large data sets.

The best initial management is to give the couple full information about the risks to the child and then for the couple to decide whether to proceed or not. However, in the situation where both partners are known to carry defects (for example, cystic fibrosis mutations) there can be up to a 50% chance of the birth of a child who will develop clinical cystic fibrosis and die young after a number of years of morbidity. In this situation, many clinicians and infertility clinic personnel may feel that their duty of care to the future child and the interests of society as a whole outweigh the wishes of the individual couple and that it is not ethical to proceed and that ICSI should not be offered to the couple. In some countries, law may govern these matters and then there is no choice, but, in the absence of law, this type of conflict makes the doctors role very difficult. Each case has to be judged on its merits and in the context of what is available and affordable in the local health care system. When there is a conflict that cannot be resolved by agreement, the interests of a future child probably take precedence over the interests of a couple. If the decision is taken to proceed, it is important for the couple to fully appreciate what may be in store for a future child and it is often appropriate for arrangements to be made for the couple to visit another family where there is a teenager or older person suffering from...
Table 4 Risk of germline therapy

<table>
<thead>
<tr>
<th></th>
<th>Uncertainty and risk</th>
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<tr>
<td>2</td>
<td>The slippery slope particularly in regard to genetic enhancement</td>
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<tr>
<td>3</td>
<td>The lack of consent by future generations</td>
</tr>
<tr>
<td>4</td>
<td>Inappropriate allocation of healthcare resources</td>
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<tr>
<td>5</td>
<td>Intrinsic immorality</td>
</tr>
</tbody>
</table>

the condition. Also, the couple will need to give consideration to pre-implantation diagnosis and replacement only of normal embryos or, if this is not available, amniocentesis and genetic diagnosis and the possibility of termination.

ICSI is new technology, but perhaps the greatest potential application is the opportunity for germ line therapy. At present in most countries this is considered unethical and sometimes illegal. Nevertheless, the following hypothetical situation may help focus debate. Suppose a couple decide to go ahead with ICSI in a situation where both partners are known to have cystic fibrosis mutations, but after successful pregnancy would not contemplate amniocentesis and abortion. If, in this situation, it was possible to correct the mutation before embryo replacement, which would be less harmful – to repair the defect and enable the birth of a child without cystic fibrosis, or not to repair the defect and for a child to be born with cystic fibrosis? These arguments can be extended to correction of oncogenes and other defects. The risks of germ line therapy (Table 4) have been summarized by Fiddler and Pergament in a review article, which gives powerful argument in favour of germ line therapy.

Summary

Our new knowledge of the genetic basis of infertility and the advent of ICSI necessitates good understanding of genetics by clinicians and the public at large. In this chapter, the emphasis is on the description of those genetic disorders more likely to be encountered by the infertility clinician. Advances in genetic diagnosis will cause discovery of the genetic basis of more disorders and easier and cheaper diagnosis of known disorders; for some of these genes, therapy may become practicable. We live in the age of genetic discovery and infertility clinicians will need to keep up-to-date with fast moving scientific advances. It will also be important that infertility clinicians keep in mind the fundamental ethical principles of beneficence, autonomy and justice and to be able to manage situation where these principles may be in conflict.
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