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Review

The genetic basis of infertility

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Infertility is defined as the inability to conceive after one year of regular unprotected intercourse; approximately one in six couples wishing to start a family fall into this category. Although, in many cases, the diagnosis is simply 'unexplained', a variety of reasons including lack of ovulation, mechanical stoppage, sperm deficiencies and parental age have been implicated. It is difficult to assess accurately the overall magnitude of the contribution of genetics to infertility as most, if not all, conditions are likely to have a genetic component, for example susceptibility to infection. Nevertheless, a significant number of infertility phenotypes have been associated with specific genetic anomalies. The genetic causes of infertility are varied and include chromosomal abnormalities, single gene disorders and phenotypes with multifactorial inheritance. Some genetic factors influence males specifically, whereas others affect both males and females. For example, chromosome translocations affect both males and females, whereas Klinefelter syndrome and the subsequent infertility phenotype caused by it are specific to males. This article reviews current research in the genetic basis of infertility; gender-specific disorders and those affecting both sexes are considered.

Infertility is defined as the inability to conceive after one year of regular unprotected intercourse and accounts for one in six couples wishing to start a family. A healthy young couple in their mid-twenties has only a 20–25% chance of establishing a pregnancy in each cycle and thus a range of factors, each with different extents of genetic control, may influence their chances. Infertility can be hormonal, related to age, exercise, obesity or infectious disease; it can be immunological, psychological, result from surgery or blockage, or be associated with defined abnormalities in the gametes (for example aberrant semen parameters). Perhaps the most common 'cause' of infertility is simply 'unexplained' and this accounts for about 20% of couples (Uehara *et al.*, 2001). It is difficult to assess accurately the genetic contribution to reduced fertility as most, if not all, of the above factors are likely to have a genetic component. For instance, susceptibility to infection, obesity and psychological problems and even the likelihood of having surgery may have some genetic basis, however small. Nevertheless, specific genotypes and karyotypes have been associated with infertility phenotypes and studies of specific genes in humans and model systems shed light on the nature of the polygenic and multifactorial basis of infertility.

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Chromosome disorders: structural aberrations

Translocations

Reciprocal translocations can lead to reduced fertility, spontaneous abortions or birth defects, depending on the chromosomes involved and the nature of the translocation. In autosome–autosome translocations, reduced fertility is mediated by the fact that the translocated chromosomes, in order to progress through meiosis, need to synapse through a pairing cross. This process can lead to infertility in several ways. First, the mechanics and time constraints imposed on the formation of such a quadrivalent can impede the meiotic process (Forejt, 1982). Second, the disjunction of the pairing cross is prone to the production of genetically unbalanced gametes. Third, asynaptic regions are common within the pairing cross and can lead to failure of meiosis and subsequently elimination of germ cells (Miklos, 1974). Fourth, there is evidence that translocated segments of chromosomes attempt non-homologous pairing with X and Y chromosomes during meiosis I (Lyon, 1966), which interferes with X inactivation, resulting in a lethal gene-dosage effect on the germ cells (Forejt, 1982). Finally, the interactions of the translocation chromosomes with other parts of the nucleus may produce errors in meiosis and cell death (Chandley, 1984). Males with reciprocal X-autosome translocations can present with

1470-1626/2003

severe spermatogenic arrest which frequently results in azoospermia (Madan, 1983). In females, the translocated X segment containing X-genetic loci is presumed to remain transcriptionally active because the X-inactivation elements have been removed from control of the translocated segment. The abnormal X inactivation of the translocated segment interferes with the genetic control of germ-cell progression, resulting in meiotic arrest at the primary spermatocyte stage (Lifschytz and Lindsley, 1972). Reciprocal Y-autosome translocations may result in aberrant spermatogenesis due to abnormal sex chromosome pairing. The most common Robertsonian translocation observed in infertile males is t(13q14q). Meiotic studies of infertile carriers of t(13q14q) and t(14q21q) reveal abnormal behaviour of the rearranged autosomes in meiosis during spermatogenesis causing infertility (Luciani *et al.*, 1984; Rosenmann *et al.*, 1985). The heterochromatic short arms of acrocentric chromosomes carry the nucleolar organizer regions (NOR) which, in addition to their function in rRNA synthesis, are required to associate with the sex vesicle. Thus, Robertsonian translocations that have lost their NOR can increase the likelihood of cell disruption and germ-cell death, thus decreasing fertility (Robez, 1986).

Chromosomal inversions

Inversions cause infertility by the following mechanisms. First, the mechanics and time constraints imposed by the meiotic machinery on the formation of a pairing loop can impede meiosis (Forejt, 1982). Second, single-sperm PCR studies of crossing over indicate that recombination is reduced within the pairing loop and this also leads to a breakdown of meiosis (Brown *et al.*, 1998a). Third, when crossing over occurs in the pairing loop, unbalanced gametes can then ensue (Chandley *et al.*, 1987).

Supernumerary and marker chromosomes

Supernumerary chromosomes are extra chromosomes that are not easily identified by normal cytogenetic means. Carriers of marker chromosomes are at risk of infertility due to meiotic arrest and instability (Chandley, 1984).

Chromosome disorders: constitutional aneuploidy

Klinefelter syndrome (47,XXY)

Klinefelter syndrome (one in 1000 males) is generally associated with the karyotype 47,XXY which can be in all cells (full-blown trisomy) or in 'mosaic' form. There are various extents of spermatogenic failure but males are generally sterile (Rives *et al.*, 2000) unless they are mosaic. Even in cases of apparent full-blown trisomy, functioning germ cells have been shown to be XY (hence the patients are gonadal mosaics) and there is compelling evidence that the compromised testicular environment in these men leads to increased chromosome segregation errors. Hence, males with Klinefelter syndrome who reproduce are more likely to have aneuploid offspring (Mroz *et al.*, 1999). The extra X chromosome originates in paternal meiosis I non-disjunction of the XY bivalent in > 50% of cases, about 40% in maternal meiosis I or II and, in the remainder, post-zygotically (Hassold *et al.*, 1996). There is an association of increased maternal age in maternal meiosis I cases (Hargreave, 2000). Griffin *et al.* (1995) found a significant increase in the incidence of XY sperm disomy in relation to age, indicating that older men, like older women (but to a lesser extent), have an increased likelihood of producing 47,XXY offspring. The incidence of XY sperm disomy or paternally derived Klinefelter syndrome is associated with a failure of the X and Y to recombine during meiosis I (Hassold *et al.*, 1991).

47,XYY males

Present in one in 1000 male births, 47,XYY arises through paternal meiotic II non-disjunction of the Y chromosome. This causes an aberrant hormonal balance in the gonadal environment which affects normal function of human chorionic gonadotrophin (Attanasio *et al.*, 1982). Some spermatogenic impairment is usual (Skakkebaek *et al.*, 1973). As with 47,XXY, fertile 47,XYY men are believed to be gonadal mosaics. This indicates that the loss of one Y chromosome, followed by a process of germ-cell-line competition, in which XY cells have a selective advantage over aneuploid cells, is responsible for normal spermatocyte development (Melnyk *et al.*, 1969).

Turner syndrome

A missing X chromosome (45,X) is the characteristic karyotype in Turner syndrome patients (frequency of about one in 5000 to one in 10 000) and occurs in about 55% of cases (Steven *et al.*, 1999). Streak gonads with hypoplasia and sexual infantilism due to the haplo-insufficiency of XY homologous genes crucial for gonadal development ensure complete sterility in most patients (Witters *et al.*, 2001). The single X chromosome is maternal in origin in 75% of cases. Thus, the predominant origin of 45,X is a spermatozoa with a missing sex chromosome and paternal meiotic non-disjunction at meiosis I or II or anaphase lag are the possible mechanisms for this (Hassold *et al.*, 1988). In addition, double anaphase lag of the paternal X or the Y chromosome in a 46,XX or XY zygote could also result in a 45,X complement (Jacobs *et al.*, 1997). Table 1 shows the relative frequencies of the many other karyotypes associated with Turner syndrome. Deletion

Table 1. The relative frequencies of the karyotypes associated with Turner syndrome

Karyotype	Description	Frequency of Turner cases (%)
45,X	One X chromosome missing	55
46,X,i(Xq) (or one of its variants)	Isochromosome X most commonly seen in mosaic form together with a 45,X lineage	20
Various structural	For example deletions and ring X chromosomes	10
46,XX/45,X	Mosaic Turner or normal	10
Various Y mosaics	Mosaicism with one cell line having a normal Y or structurally rearranged Y chromosome	5

of Xp produces a greater number of Turner features than does deletion of Xq.

47,XXX

Occurring in one in 1000 females, in 95% of cases the extra X chromosome is maternal in origin and associated with increased maternal age (Hassold *et al.*, 1996). Most 47,XXX females are of normal weight, height and mental function, have normal pre-pubertal development and are fertile but have an early onset of menopause at about 30 years of age compared with the average of about 50 years of age (May *et al.*, 1990). Increased dosage of genes that escape X inactivation accounts for clinical features and individuals with four or more X chromosomes have been reported. Severity of symptoms increases in proportion with the number of X chromosomes.

Down syndrome (trisomy 21)

Trisomy 21 is the leading cause of mental retardation in humans and occurs in an estimated one in 700 births. Affected females in rare cases can reproduce; however, most if not all, affected males are sterile: the phenotype includes spermatogenic arrest, reduction in the number of germ cells and hylinized tubules. The mechanism by which trisomy 21 affects male infertility remains unclear, but it is hypothesized that it may be due to a reduced proliferation of primordial germ cells during migration to the gonadal ridge, perhaps associated with an accelerated rate of apoptosis (Patrizio and Broomfield, 1999).

Chromosome disorders: aneuploidy in the gametes

Maternal age effect for aneuploidy

The most commonly cited relationship between genetics and reduced fecundity in women is the maternal age effect. Although usually discussed in terms of the risk of producing trisomic offspring (for example Down syndrome), the risk of trisomy associated with age is also the single most common factor in pregnancy loss. That is, approximately 25% of all first trimester spontaneous abortions are trisomic and it is likely that many other aneuploid conceptuses (for example the autosomal monosomies and trisomies of chromosomes 1 and 19) are lost before they reach the stage of clinical recognition. The dogma purported in many genetic texts to explain the maternal age effect is the so-called Production Line Hypothesis. This hypothesis, on the basis of observations by Henderson and Edwards (1968) in rabbit eggs, indicates that oocytes entering meiosis first are the first to be ovulated and those entering last are ovulated last. It is hypothesized that oocytes entering meiosis last are more prone to non-disjunction. However, a confounding issue is the fact that mammalian eggs enter meiosis and proceed to diplotene prenatally and only resume meiosis upon ovulation. In the current authors' opinion (and that of other authors) it is biologically implausible that events initiated before birth can have such profound effects on chromosome segregation many years later (for example, see Speed and Chandley, 1983; Griffin, 1996; Koehler *et al.*, 1996). An alternative is the Local Factors Hypothesis (Crowley *et al.*, 1979; Sugarawa and Mikamo 1983; Eichenlaub-Ritter *et al.*, 1988), which indicates that extrinsic factors in the ovary associated with ageing affect the egg directly. That is, the ovarian environment becomes compromised as the woman ages and eggs within it become progressively less able to disjoin chromosomes normally. Changes in, for example, oxygen concentration, pH or hormone concentrations (all associated with ageing) have been implicated as subsequently affecting chromosome segregation in later meioses (Gaulden, 1992). Indeed, Van Blerkom and co-workers demonstrated that the spindle apparatus is less well-formed in eggs from older women which have a lower intracellular pH and are present in a more hypoxic environment (Van Blerkom *et al.*, 1997; Van Blerkom 1998, 2000; Van Blerkom and Davis, 2001). The work of Hawley and co-workers has extended this hypothesis further by comparisons of non-disjunction of human chromosome 21 and the *Drosophila* NOD^{DTW} mutation (Hawley *et al.*, 1994; Koehler *et al.*, 1996). This led to the hypothesis that older eggs are less likely than younger eggs to segregate properly bivalents with distal chiasmata (or no chiasmata at all), whereas both older and younger

oocytes have less difficulty segregating bivalents with proximal (or two) chiasmata. It is also hypothesized that a human homologue of the NOD gene product (a kinesin-like protein involved in maintaining contact between homologous chromosomes and between centromeres and kinetochores) plays a central role. However, to date, the lack of human material and few comparable mammalian systems have impeded the testing of this and other hypotheses including the Limited Pool Hypothesis (Peters and McNatty, 1980), which proposes that the depletion of oocytes in the ovary leaves the remainder more prone to non-disjunction.

High proportions of chromosomally abnormal spermatozoa in infertile men

Numerous authors have reported a correlation between the proportion of aneuploid spermatozoa (that is, spermatozoa with an extra or missing chromosome) in an ejaculate and severe defects in the conventional parameters of semen quality. That is, fluorescent *in situ* hybridization (FISH) studies indicate that severely infertile males may have 70% or more spermatozoa that are aneuploid (Bernardini, 1997; Lahdetie *et al.*, 1997; Aran *et al.*, 1999; Pang *et al.*, 1999; Pfeffer *et al.*, 1999; Shi and Martin, 2000; Ushijimal *et al.*, 2000; Calogero *et al.*, 2001). However, there have been reported cases where no such relationship is observed (for example Guttenbach *et al.*, 1997). These discrepancies between studies may be due to differences among laboratories in scoring criteria but another possibility is that intrinsic (for example age or DNA polymorphisms) or extrinsic (for example environmental pollutants) factors play a role. Indeed, cigarette smoke, alcohol and chemotherapy regimens all cause increased sperm aneuploidy, whereas age has been clearly shown to be associated with increased sex chromosome sperm aneuploidy (for example Griffin *et al.*, 1995; Robbins *et al.*, 1997). It is hypothesized that the reduction or absence of recombination between the X and Y chromosomes is a common mechanism that explains the correlation between XY disomy and oligozoospermia (H. Tempest, D. Christopikou, M. Dalakiouridou, X. P. Zhai, S. Homa and D. K. Griffin, unpublished). Men with severely compromised semen parameters are usually treated by intracytoplasmic sperm injection (ICSI), which bypasses the normal barriers to fertilization. It is hypothesized that men treated by ICSI have an increased risk of producing trisomic offspring, especially for the sex chromosomes. Collectively, studies with patients who undergo ICSI have reported that 15 in 2084 patients show sex chromosome abnormalities; this is 5–10 times the published population frequency. However, no such associations have yet been reported for autosomal trisomy (Hassold *et al.*, 1996; Bonduelle *et al.*, 1999, 2002). Therefore, the question of whether males should be screened for sperm aneuploidy before treatment with ICSI is the topic of considerable debate (Griffin *et al.*, 2003).

Y chromosome genes and microdeletions affecting fertility

The AZF (azoospermia factor) region Yq11 contains genes vital for spermatogenesis. Vogt *et al.* (1996) and Affara *et al.* (1999) subdivided this region into AZFa, AZFb and AZFc. Deletions within these sub-regions cause various spermatogenic and infertility phenotypes (Affara and Mitchell, 2000) and represent about 10–15% of idiopathic azoospermia and oligozoospermia. For the most part, deletions in the AZFa region lead to Sertoli-cell only syndrome and azoospermia (or severe oligozoospermia). Deletions of AZFb and AZFc have a more diverse range of phenotypes.

Ma *et al.* (1993a,b) isolated AZFb genes (*RBM 1* and *2*) that were found to contain an RNA recognition motif and have homology to the RNA-binding protein superfamily. The absence of these genes leads to defects in RNA metabolism and processing. There are at least six sub-classes of human *RBM* on the Y chromosome (*RBMY1–RBMY6*) but only the *RBMY1* sub-class is transcribed (Chai *et al.*, 1997). The *RBMY* protein is localized to the nucleus, confined to germ cells, and also appears to be present at all stages of spermatogenesis except in elongating spermatids (Elliott *et al.*, 1997).

In AZFc, the gene *DAZ* (deleted in azoospermia) contains an N-terminal RNA binding motif and a series of repetitive amino acids (Reijo *et al.*, 1995). This finding indicates a role for *DAZ* as an RNA-binding protein concerned with RNA metabolism but its exact function, to the best of the current authors' knowledge, is yet unknown (Ferlin *et al.*, 1999a). It is thought to be important in the control of spermatogenesis, as deletion of *DAZ* is widely prevalent among infertile men. Moreover, there are now thought to be at least three copies of this gene within AZFc. It is difficult to determine the exact function of each copy; indeed it is uncertain whether each copy is expressed, because usually the whole cluster is deleted in infertile men. An autosomal homologue of *DAZ* (*DAZL1*) has also been found in humans on chromosome 3p; *DAZL1* is expressed specifically in the testis and (to a lesser extent) in the ovaries (Saxena, 1996) and could be responsible for autosomal recessive forms of male infertility (Affara and Mitchell, 2000). Its role in spermatogenic control is supported by its homology to the *Drosophila* male infertility gene *Boule*, which, when mutated, causes spermatogenic arrest (Reijo *et al.*, 1995; Ferlin *et al.*, 1999a). The protein product is found mainly in post-meiotic germ cells affecting post-transcriptional control during the transcriptionally inactive stages of germ-cell differentiation. The *DAZL1* protein in mice is expressed in the cytoplasm of the spermatocytes in pachytene, leptotene and zygotene stages but is not seen

Box 1. Summary of observations in men with deletions in the azoospermic factor (AZF) region

Microdeletions are found almost exclusively in males with azoospermia or severe oligospermia
A higher frequency of deletions is seen in azoospermic men
Large deletions are generally associated with more severe spermatogenic defects
AZFa deletions (relatively uncommon) are generally associated with SCO (Sertoli-cell only syndrome)
AZFb and AZFc deletions (most common) may be associated with a variety of defects

in post-meiotic mouse germ cells (Ruggiu *et al.*, 1997). Habermann *et al.* (1998) suggest that the *DAZ* transcripts are not immediately translated but are complexed to a non-translatable ribonucleoprotein fraction and stored until after meiosis and involved in transporting mRNA to various parts of a developing spermatozoon.

Deletions within the AZFa interval occur at a much lower frequency than for AZFb and AZFc. Candidate genes for infertility within this region include *DFFRY*, *DBY* and *UTY* (Mazeyrat *et al.*, 1998; Ferlin *et al.*, 1999b; Affara and Mitchell, 2000; Foresta *et al.*, 2000). All three genes have ubiquitously expressed X homologues. *DFFRY* (*Drosophila* fat facets related (on Y chromosome)) encodes a ubiquitin-specific hydrolase which is involved in oocyte development in *Drosophila* (Brown *et al.*, 1998b; Sargent *et al.*, 1999) and is also involved in ubiquitin-dependent degradation of protein and specifically targets a transcription factor (D-jun) and other members of the signal transduction cascade involved in regulating (D-jun) activity. *DBY* (DEAD box Y gene) encodes a protein containing the DEAD box motif found in RNA helicases and therefore may be involved in RNA metabolism. The function of *UTY* (ubiquitous transcribed tetrapeptide repeat gene on Y chromosome) is currently unknown (Odorisio *et al.*, 1996) but deletions in this gene have been found in both fertile and infertile men. It is in this region that the only point mutation on the Y chromosome that is associated with infertility has been found: a four-base pair deletion in the splice site of *DFFRY* (Sun *et al.*, 1999). Indeed, the predominant lesions in Y chromosome are large deletions. The reasons for this are thought to be the large numbers of repeat sequences on the chromosomes causing unequal sister chromatid exchange (Blanco *et al.*, 2000; Kuroda-Kawaguchi *et al.*, 2001). This can lead to progressive rearrangements of the Y chromosome, a variable phenotype over time (or age effect) and subsequent problems in establishing genotype or phenotype correlations. The observations that have been made in men with deletions in the AZF region oligoszoopermia are summarized in Box 1. In general terms it is thought that differential removal of a range of genes leads to the different spermatogenic phenotypes. Indeed, many genes may be present in several copies and thus differential deletion of them may lead to dosage effects (Vogt *et al.*, 1997; Affara and Mitchell, 2000).

The CFTR gene and fertility

Cystic fibrosis and congenital bilateral absence of the vas deferens

Cystic fibrosis (CF) is the most common fatal autosomal recessive disorder in Caucasians, with an incidence of one in 25 and a carrier frequency of one in 2400. It arises because of mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*; Hargreave, 2000). Infertility in males with a *CFTR* mutation is found to be due mainly to obstructive azoospermia (Sheynkin, 2000). The gene 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. The *CFTR* gene is expressed in the postnatal human epididymis, with the protein found in the luminal border of the human cauda epididymal epithelium. The CFTR protein is also involved in the transport of electrolytes and water across the epididymal epithelium, which helps to achieve an optimum environment for sperm maturation and transport (Pallares-Ruiz, 1999). This involvement in sperm maturation indicates that mutations in the *CFTR* gene may cause infertility not only through obstruction but also through deficiencies in sperm maturation. Genetic studies have revealed that 50–83% of patients with congenital bilateral absence of the vas deferens (CBAVD) have at least one known *CFTR* gene mutation and that approximately 10% have two known *CFTR* mutations (Donat *et al.*, 1997). More than 95% of men with CF have abnormalities in structures derived from the Wolffian duct. Although CBAVD is genetically similar to CF it is a clinically distinct disorder. It is found in 2% of men who present with infertility and is more commonly associated with obstructive azoospermia, low semen volume and acidic pH (Heaton and Pryor, 1998; Meschede *et al.*, 1998). The most common cause of CBAVD seems to be a combination of the 5T allele in one copy of the *CFTR* gene which lacks exon 9, resulting in low expression of functional CFTR protein (Chu, 1991), and a CF mutation, the most common being ΔF508, in the other copy (Chillon *et al.*, 1995). *CFTR* mutations have been found in some men with congenital unilateral absence of the vas deferens, and this has led Chillon *et al.* (1995) to suggest that it could be an incomplete form of CBAVD. Men with this disorder appear to have normal fertility and are rarely diagnosed (Meschede *et al.*, 1998).

Table 2. Genes implicated in infertility

Gene	Proposed function	Role in infertility	Comments	Reference
CFTR	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	Involved in congenital bilateral absence of the vas deferens, congenital unilateral absence of the vas deferens, bilateral ejaculatory duct obstruction and Young syndrome	See text	Heaton and Pryor, 1990; Chu *et al.*, 1991; Hirsh *et al.*, 1993; Chillon *et al.*, 1995; Meschede *et al.*, 1997, 1998; Pallares-Ruiz, 1999; Sheynkin, 2000; Meng *et al.*, 2001
KALIG-1	Encodes a protein with homology to neural cell adhesion molecule, allowing normal migration of GnRH neurones from their site of embryonic origin in the medial nasal placode to the hypothalamus	Prevents the movement of GnRH to the hypothalamus during development[8] and subsequent testis failure. X-linked form of Kallman syndrome results from a mutation in this gene	Variable penetrance regardless of the inheritance pattern	Nudell and Turek, 2000
PTPN11	Encodes two Src homology 2 (SH2) domains[33]. The protein encoded is present in an active and inactive conformation	Responsible for just over half the cases of Noonan syndrome	Mis-sense mutations have been found to affect the amino terminal SH2 domain and the phosphotyrosine phosphatase domains, resulting in the protein being continually active. Therefore, Noonan syndrome is due to over activity of the proteins[33]	Tartaglia *et al.*, 2001
SF1	A member of the nuclear receptor family that regulates expression of steroid hydroxylases[34]	*SF1* XY knockout mice do not have adrenal glands and gonads. A mis-sense mutation in the DNA binding domain of *SF1* results in an XY individual exhibiting streak gonads and fully developed Mullerian structures.[37] Possibly in the Leydig cells where it regulates production of steroid hormones[36]	Resembles the *fushi tarazu* gene, a *Drosophila* nuclear receptor gene involved in development. Not necessary for initial gonad development but expressed during testis differentiation[34]. Possibly *e*xpressed in the bipotential gonad and Sertoli cells regulating the expression of anti-Mullerian hormone	Parker, 1998; Roberts *et al.*, 1999; Swain and Lovell-Badge, 1999; Veitia *et al.*, 2001
SOX9	A member of a family of transcription factors that contain an *SRY*-related HMG box (*SOX*)	Mutations in *SOX9* gene have been found in individuals who are chromosomally male but phenotypically female. The SOX9 protein is found in the bipotential gonads then is not seen thereafter in the developing ovaries but is present in the fully developed male gonads	Mouse transgenic studies show that female-to-male sex reversal occurs when female mice carry *Sox*9. Expression is upregulated in the Sertoli cells shortly after *Sry* expression, indicating that *Sox9* is activated by *SrY*	Clarkson and Harley, 2002

Table 2. (Continued)

Gene	Proposed function	Role in infertility	Comments	Reference
DAX1	A member of the nuclear hormone receptor superfamily	Thought to be responsible for congenital adrenal hypoplasia and dosage-sensitive sex reversal (caused by an Xp duplication) in humans. *Dax1* proteins are present in the early genital ridge of both males and females. Expressed at the same time as *Sry*[3]	*Sry* and *Dax1* are antagonistic to each other. Also interacts with *SF1*, forming heterodimers with *SF1* and thereby preventing *SF1* from binding and activating its target genes	Swain *et al.*, 1998 Veitia *et al.*, 2001
WT1	A zinc-finger protein that functions as a transcription factor[44]	Associated with Denys–Drash syndrome and Frasier syndrome	In Frasier syndrome XY males develop as females and donor splice mutations have been found	Swain and Lovell-Badge, 1999
GATA4	GATA proteins are zinc-finger proteins involved in transcription that recognize a consensus DNA sequence, (A/T) GATA(A/G) (the GATA motif)[45]	Expressed in bipotential gonads of both sexes and regulates AMH in males after differentiation of the testis. Expression in males continues through to adulthood. Expressed in the Sertoli cells during fetal and post-natal development. Also expressed in the Leydig cells until puberty when it shows an increase in expression that coincides with testosterone production	Increased expression during FSH and testosterone production indicates that androgen action could influence the expression of *GATA4*. When testes are treated with GnRH, there is an increase in the amounts of *GATA4* mRNA production	Swain *et al.*, 1998 Swain and Narvez, 1998
WTN4	Encodes secreted glycoproteins, rich in cysteine residues that function as signalling molecules. Involved in transcriptional activation	Deletions have been found to cause male sexual development in XX mice. Over-expression in humans is thought to result in XY sex reversal[49]	During sex determination in humans *WNT4* upregulates the expression of *DAX1*	Jordan *et al.*, 2001 Vilain, 2002
DMRT1	Male-specific gene that encodes a protein with a conserved zinc-finger-like DNA-binding domain (the DM domain)	Expressed in the genital ridge of both sexes during early development but to a greater extent in males. Mice heterozygous for *Dmrt1* have normal testes and are fertile but those homozygous for the mutant *Dmrt1*, missing the DM domain, show incomplete testis development	In mice, *Dmrt1* is expressed in spermatogonia, thereby indicating a role in the meiotic or mitotic cell cycle. In mice with mutations in *Dmrt1*, the Sertoli cells have abnormal morphology, over-proliferate and die	Raymond *et al.*, 2000 Zarkower, 2001

Table 2. (Continued)

Gene	Proposed function	Role in infertility	Comments	Reference
Dhh–Ptch1	*Dhh–Ptch1* protein complex plays a role in gonad differentiation	May be involved in the origin of the fetal Leydig cells	The *Dhh/Ptch1* signal upregulates the expression of steroidogenic factor 1 (*SF1*) and P450 side-chain cleavage enzyme resulting in Leydig cell differentiation	Yao *et al.*, 2002
Fertilinβ	Originally described in guinea-pigs as PH-30, fertilin complex consists of an α and αβ subunit and is involved in sperm–egg binding and fusion	The β subunit is thought to bind to an oocyte vitelline membrane receptor which, along with the α subunit, mediates membrane fusion and allows fertilization to take place		Burkin *et al.*, 1997
AKAP 82	A kinase anchoring protein family of polypeptides, believed to be involved in signal transduction system which brings about the co-ordinated and integrated movement of the axoneme	Possible defects within this pathway may result in immotile sperm phenotype as seen in asthenozoospermia	Studies have also demonstrated that *AKAP 82* is present in the fibrous sheath of sperm tails; this becomes tyrosine phosphorylated during sperm capacitation. Therefore it is possible that this may also link fertilization competence with motility	Colledge and Scott, 1999

Bilateral ejaculatory duct obstruction

Bilateral ejaculatory duct obstruction (BEDO) is usually caused by infections, cysts or trauma. In cases with no obvious external cause *CFTR* mutation can occur. Meschede *et al.* (1997) carried out an analysis of the *CFTR* gene in a group of seven men with BEDO. They found that five were compound heterozygotes for either two different mutations or for a single mutation and a 5T allele. One was heterozygous for the R347P mutation.

Young syndrome

Young syndrome can cause a complete bilateral plugging of the epididymal lumen and result in azoospermia (Meschede *et al.*, 1998). There is a single report that the prevalence of *CFTR* mutation ΔF508 was slightly higher in men with Young syndrome, though analysis of another group of men with Young syndrome did not provide evidence of any *CFTR* mutations (Hirsh *et al.*, 1993; Meschede *et al.*, 1998).

Other genes implicated in infertility

A list and comment on a number of genes that have been implicated in infertility is provided in Table 2. Many of these genes have been isolated through model systems, for example in mice. Clearly, these model systems are essential tools to understand further the multifactorial nature of infertility. Table 2 highlights genes that, in general, have appeared frequently in the literature; however, to the best of the authors' knowledge, over 200 are now reported (Matzuk and Lamb, 2003). A more complete list can be found at http://www.nature.com/fertility.

Of particular interest is the androgen receptor gene in which a number of studies have reported an association between the length of the CAG repeat in exon 1 of the gene l and male infertility. For instance Casella *et al.* (2003) report an association between males with testicular failure (especially those with hypospermatogenesis) and a significantly elongated androgen receptor polyglutamine tract compared with controls. This is also reported in previous studies by, for example Misfud *et al.* (2001) and Yoshida *et al.* (1999). However, this finding has been challenged by a number of authors (for example Rajpert-De Meyts *et al.* (2002) and Van Golde *et al.* (2002)) who found no such association. Clearly, further work needs to be carried out to clarify the nature of any association.

Further syndromes with a genetic basis affecting fertility

Prader-Willi syndrome and Angelman syndrome

Prader-Willi syndrome is characterized by short stature, obesity, hypotonic muscles, mental retardation

Table 3. Genetic syndromes associated with infertility

Syndrome	Comments	Reference
Non-classical adrenal hyperplasia	Women have an increased risk of having children with genital abnormalities or life-threatening metabolic crises if the father is a carrier	Hickey *et al.*, 2002
Kallmann syndrome	Also known as hypogonadotropic hypogonadism, can be sporadic or inherited in an X-linked, autosomal dominant and autosomal manner. A heterogeneous genetic disorder affecting one in 10 000–60 000. Males predominate in a ratio of 5 : 1 and the X-linked form results from a deletion in the *KALIG-1* (Kallmann interval 1) gene (Table 2)	Nudell and Turek, 2000
Immotile cilia syndrome	A recessive disorder resulting in impaired or absent ciliary or flagellar motility. Characterized by defects in both the microtubule and dynein arm assembly and is found in both ciliary and sperm tail axonemes	Nudell and Turek, 2000
Noonan syndrome	An autosomal dominant disorder, similar to Turner syndrome. Common features include short stature, pulmonic stenosis, webbed neck testicular atrophy and cryptorchidism affecting one in 1000–2500. A heterogeneous syndrome with one gene (protein–tyrosine phosphatase, non-receptor-type 11 (*PTPN11*)) so far discovered (Table 2)	Tartaglia *et al.*, 2001
Denys–Drash syndrome (DDS) and Frasier syndrome	DDS is characterized by severe genito–urinary malformations. Males with DDS seem to be more severely affected than females exhibiting ambiguous or even female genitalia. Heterozygous mutations in *WT1* have been implicated (Table 1). In Frasier syndrome, another disorder characterized by mutations in the *WT1* gene, XY males develop as females and donor splice mutations have been found	Swain and Lovell-Badge, 1999
Androgen insensitivity syndrome	Also known as testicular feminization, there are a number of different forms including non-sense mutations, point mutations and splice variants. Features include defective spermatogenesis oligozoospermia, azoospermia and testicular atrophy as a result of the dysfunction of the androgen receptor to the action of circulating androgens affecting one in 60 000. An X-linked recessive syndrome	Patrizio and Broomfield, 1999b
Polycystic kidney disease	Affected patients present with multiple cysts in the liver, kidneys, epididymis and seminal vesicles, affecting one in 800. An autosomal dominant syndrome 16p13.3 PKD1	Patrizio and Broomfield, 1999c
Ushers syndrome	Most common cause of inherited deafness and blindness, some patients also present with degeneration of the sperm axoneme. Studies have revealed abnormal microtubular organization of the axoneme in a number of cells including sperm tails resulting in poor motility, and affecting an estimated one in 30,000. Autosomal recessive syndrome	Patrizio and Broomfield, 1999d

and hypogenitalism. These characteristics are caused by small deletions of an imprinted region of proximal chromosome 15q of paternal origin or maternal uniparental disomy for chromosome 15 (Lalande, 1996). Most individuals with Prader-Willi syndrome do not reproduce. In Angelman syndrome (caused by deletion of the same region of maternal 15 paternal uniparental disomy 15), successful reproduction has been reported in only one case (Lossie and Driscoll, 1999).

Polycystic ovarian syndrome

Polycystic ovarian syndrome (PCOS) is associated with insulin resistance, defects in insulin secretion and a substantial risk of developing Type 2 diabetes mellitus. Defects in insulin-mediated receptor autophosphorylation have been found in a substantial proportion of women. Studies of 50 families of PCOS probands (Legro *et al.*, 2002) indicate that 24% of sisters are affected with PCOS. There also appears to be an intermediate phenotype of sisters with regular menstrual cycles who are hyperandrogenic (22% of sisters).

Myotonic dystrophy

Mytotonic dystrophy (DM) is present in one in 10 000 and the clinical features, in particular the variable age of onset and mode of inheritance (anticipation) are well described. The gene on chromosome 19 codes for a regulatory protein kinase found in skeletal muscle. Mistakes in faithful copying of the gene during gametogenesis result in the amplification (more rapid in male transmission) of a CTG triplet repeat and account for the variable age of onset and increased severity in subsequent generations (Brunner *et al.*, 1993). Females with DM have clinical hyperandrogenism, which affects the hormones that control fertility; younger women are less severely affected (Cordray *et al.*, 1994). Fertility of DM males can also be affected; Hortas *et al.* (2000) report that this is due to deficient capacitation and acrosome reactions in spermatozoa of DM patients. Hauser *et al.* (1991) also report that DM causes sclerosis of the tubuli seminiferi contorti, which can ultimately lead to azoospermia.

Other syndromes

Further genetic syndromes associated with infertility include non-classical adrenal hyperplasia, Kallmann syndrome, immotile cilia syndrome, Noonan syndrome, Denys–Drash syndrome and Frasier syndrome. These syndromes are summarized in Table 3.

Conclusions

Infertility is a broad term used to define a range of different phenotypes. The genetics of infertility is very complex and is dependent on different factors. Genetic factors can affect the production of the germ cells, the ability of the gametes to meet or embryonic development. Genetic disorders can be chromosomal, involve single genes or be multifactorial; however, these terms are not mutually exclusive. For instance, there are presumably many genes to be discovered that are involved in chromosome malsegregation associated with the maternal age effect. Perhaps studies of infertile men with high levels of sperm aneuploidy might shed further light on these. Reproduction is clearly a subject of intense interest and much research. There is great potential for new drug therapies and the improvement of current drug therapies. Treatment for infertility is unique in medicine as it offers individuals radical invasive treatment regimens that are of little or no benefit to the health of the individual being treated. Clearly the hope is that a greater understanding of the genetic control of infertility will bring low-risk treatment regimens that are effective and easy to administer.

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