Genetic aspects of female reproduction

The ESHRE Capri Workshop Group

BACKGROUND: Sexual reproduction provides the means for preserving genetic identity and in turn, genetic variability may affect the ability to reproduce. This review aims to summarize current research on genetic diagnosis and genetic causes of reproductive disorders. METHODS: Searches were done by subject in Medline and other databases, and each subject summary was presented to the Workshop Group and omissions or disagreements were resolved by discussion. RESULTS: Single-gene defects are most likely to be found among patients with hypogonadotropic hypogonadism, which may be due to defects in the KAL genes or the gonadotrophin-releasing hormone receptor genes. With premature ovarian failure there is an increased risk of having a premutation of the Fragile X syndrome gene. Complex genetic inheritance may explain the variable familial links in polycystic ovary syndrome and endometriosis, but no definitive genetic pathways are as yet known. With recurrent miscarriage, genetic defects causing thrombophilias are 2-fold more likely. Chromosome abnormalities account for nearly 60% of all spontaneous abortions, and the most common type, trisomy, is closely associated with advanced maternal age. Three percent of couples have a balanced chromosome abnormality, but live birth rates are better with natural conception than with preimplantation genetic diagnosis. CONCLUSIONS: Understanding of the methods used for genetic diagnosis and research is becoming a standard requirement for the clinical practice of reproductive medicine.

Keywords: hypogonadotropic hypogonadism; premature ovarian failure; polycystic ovary syndrome; Rokitansky syndrome; pregnancy loss

Introduction

Genetic variability and epigenetic factors affect reproduction and fertility from gametogenesis to birth. The human genome is contained within 23 pairs of chromosomes, each containing hundreds or thousands of genes. The estimated 20,000 genes each make an average of three proteins. The genome involves three billion base pairs which are subject to countless variations that may affect health and disease (Fig. 1). Single-gene defects may cause female infertility, although this is rare (Fauser and Hsueh, 1995) more commonly complex genetic inheritance contributes to variations in the frequency of diagnoses such as endometriosis.

The four mechanisms for inheritance of disease are chromosomal, Mendelian, mitochondrial and complex.

Chromosomal disorders may be numerical, such as polysoy or polyploidy, or structural. Translocation is the most clinically important structural disorder and it may be reciprocal (exchange of two terminal segments from different chromosomes), or Robertsonian (centric fusion of two acrocentric chromosomes). Balanced translocation occurs in about 1 in 600 newborns (Jackson, 2002).

Mendelian types of heritable disease are single-gene defects which are usually autosomal dominant, autosomal recessive or X linked.

Mitochondrial inheritance: Mitochondrial DNA (mtDNA) may be 10–20 times more frequently mutated than nuclear DNA. About one-third of the oocyte DNA is mitochondrial, while the sperm mitochondria do not survive in the oocyte, and therefore mitochondrial abnormalities are transmitted only along the maternal line. Abnormalities in mtDNA are likely to cause fertilization abnormalities and disturbances in early development. In patients with mitochondrial disease, the number of mtDNA molecules that carry a mutation is increased compared with healthy carriers. Most pathogenic mtDNA mutations are heteroplasmic, that is, the mitochondrial genomes are not identical, resulting in heteroduplexes after polymerase chain reaction (PCR) amplification of mtDNA. These heteroduplexes can be detected with a technique of denaturing high performance liquid chromatography (van den Bosch et al., 2000), this is also possible via microarray technology (van Eijsden et al., 2006).

Complex genetic inheritance: Many of the differences between individuals reflect features that show continuous quantitative variability. The inheritance of such features does not follow the rules of simple, monogenic heredity. Complex genetic inheritance of disease is characterized by familial clustering and a recurrence risk which is higher than the population risk. The incidence is
always less than that with Mendelian disorders and can be estimated only by observations among families and population samples. In genetic terms the complexity results from the interaction of endogenous (genetic) and exogenous (environmental) factors. Such a pedigree pattern might also result, however, from the interaction of more than one genetic factor. Family and population data help to explain diseases with complex genetic inheritance, but heritability estimates obtained from twin studies are of special importance.

While understanding of complex genetic mechanisms is evolving slowly, knowledge of single-gene defects is expanding rapidly, in part because of new and powerful genetic investigations. When these methods are applied, a distinction has to be made between the research application (to find genes that may underlie disease) and the diagnostic application (to detect chromosome abnormalities or gene mutations in an individual patient or family).

The research methods may include longitudinal family and twin studies and in some cases ethnic studies. Candidate gene studies of genes known to be related to the disease look for differences in the frequency of genetic variants between disease cases and healthy controls, although increasingly the research involves a search across the whole genome. Genome-wide association studies can compare the frequency of up to one million single-nucleotide polymorphisms (SNPs) in cases and controls. The genetic diagnostic methods include pedigree analysis, chromosome analysis, assessment of single-gene defects and examination of the genome for disorders of gene copy number or mutations that may assist with a diagnosis.

The objectives of this review are to: outline genetic diagnostic methods; summarize the impact of chromosomal abnormalities on miscarriage; consider single-gene mutations that cause infertility; assess whether complex genetic mechanisms may influence the frequency of polycystic ovary syndrome (PCOS), endometriosis and fibroids; and summarize genetic factors that influence the risk for and the management of recurrent miscarriage (RM). Searches were done in Medline and other databases by subject and each subject summary was presented to the Workshop Group, where omissions and disagreements were resolved by discussion.

**Genetic methods for diagnosis**

Clinical practice increasingly depends on knowledge of the principles of genetics and the techniques used by geneticists. These include use of pedigrees, cytogenetic analysis, fluorescence in-situ hybridization (FISH) and methods of molecular genetics diagnosis such as PCR and comparative genomic hybridization. Table I summarizes the methods used to detect genetic mutations.

**Preimplantation genetic diagnosis**

Preimplantation genetic diagnosis (PGD) can rule out a specific genetic disease known to exist in one or both parents before pregnancy has been established. While PGD is not used to diagnose genetic causes of female infertility, it can be done only within a cycle of in vitro fertilization (IVF). One blastomere is removed from Day 3 embryos which are usually at the 6–10-cell stage of development. The blastomere is tested by PCR if there is a single-gene defect, and by FISH if there is a translocation or to exclude sex-linked disorders. Only embryos free from the known defect will be transferred to the mother (Sermon et al., 2007). The difficulties with PGD include a small diagnostic error rate (1–3%), lower live birth rates with IVF than normal conception and high cost. The effectiveness of PGD is self-evident and many couples prefer this approach to prenatal testing and possible selective termination.
Preimplantation genetic screening

Preimplantation genetic screening (PGS), in contrast to PGD, is done for couples who do not have a known genetic defect but appear to be at high risk of aneuploidy because of advanced maternal age, recurrent pregnancy loss or recurrent implantation failure. It was assumed that screening for aneuploid embryos and transferring only euploid embryos would reduce pregnancy losses and increase live birth rates. Two randomized controlled trials have shown, however, that PGS would appear not to be effective in improving live birth rates in IVF or intracytoplasmic sperm injection for women aged 35–41 years (Staessen et al., 2007). Trials are needed to determine whether PGS is effective for recurrent pregnancy loss or recurrent implantation failure.

Chromosomal abnormalities and pregnancy loss

The modern study of chromosomes in reproduction began with the discovery of the histologic distinction between the cells of males and females (Barr and Bertram, 1949). Knowledge of the Barr body gave rise to the Lyon hypothesis that in normal female cells one X chromosome is inactivated during embryogenesis (Lyon, 1961). The first report of how chromosome abnormalities contribute to fetal wastage was based on cytogenetic analysis of 200 miscarriages, of which 44 (22%) had a chromosome abnormality (Carr, 1963, 1965). The three main classes of abnormality were 45X, trisomy and polyploidy. Carr demonstrated that (i) the maternal age of miscarriages with trisomy but not the other categories was significantly increased and (ii) mean gestational age at the time of miscarriage was significantly lower for a chromosomally abnormal fetus than one that is chromosomally normal. He also suggested that the proportion of chromosome abnormalities detected was likely to be a serious underestimate.

Frequency and types of chromosome abnormality in miscarriage

Many series of karyotyped miscarriages have confirmed that ~50% of all clinically recognized spontaneous miscarriages have a chromosome abnormality. Of these, ~9% have a 45X constitution, 30% have trisomy, 10% are either triploid or tetraploid and 2% have a structural rearrangement (Table II).

Trisomy is the most common class of abnormality and trisomy frequency in miscarriages is associated with increased maternal age, rising from very low at age 20–24 up to 35% at age 40–44 years. As a result, trisomy rates are rising with the increase in mean age at childbirth. In the last 20 years in the eastern United States, the trisomy rate increased in spontaneous miscarriages from 22 to 42% as the average maternal age rose from 29 to 34 years (Table III) (Warburton, 2007).

Theoretically for each trisomy there should be a corresponding monosomy but the only monosomy that is detected among clinically recognizable pregnancies is that for the sex chromosomes. The autosomal monosomies are likely to be incompatible with survival and a major cause of pregnancy wastage in early pregnancy.

The frequency and outcome of trisomy varies greatly for different chromosomes. As can be seen from Table IV, trisomy for chromosome 16 is by far the most frequent but never survives to birth, while the next most frequent are trisomies for chromosomes 21 and 22. Only three autosomal trisomies (13, 18 and 21) and the three sex chromosome trisomies are compatible with live birth.
While the single monosomy for the sex chromosomes (XO) can be born alive the great majority are fetal deaths.

Trisomy is usually the result of non-disjunction at the first or second meiotic division, most often in the female. However, the mechanism underlying the error is, surprisingly, very different for different chromosomes. Table V shows the mechanism of non-disjunction for five different chromosomes. The X chromosome is the only one in which non-disjunction in the male makes an important contribution and this is the result of failure of recombination between the X and Y chromosomes at the first meiotic division in the male. This error gives rise to 50% of all XXY males. Non-disjunction of the X in oogenesis occurs at both the meiosis I and II, and may be nulli-chiasmate (failure of recombination) or chiasmate. All mechanisms except nulli-chiasmate are associated with increased maternal age. In contrast all trisomy 16s result from non-disjunction at the first maternal meiotic division, and are associated with increased maternal age. Trisomy for chromosome 13 is due to non-disjunction at meiosis I or II and both mechanisms are associated with increased maternal age, while trisomy for chromosome 18 is almost entirely due to non-disjunction at meiosis II, again associated with increased maternal age. Trisomy for chromosome 21 is mainly due to non-disjunction associated with abnormal recombination in meiosis I and also in meiosis II, both mechanisms being associated with advanced maternal age.

A quite different increased risk for trisomy is shown by rare individuals who have, even at a young maternal age, two or more trisomy miscarriages involving trisomy for the same chromosome. This can be the result of the mother being a gonadal mosaic for the trisomy which increases her risk of
producing oocytes carrying an additional copy of the trisomic chromosome (James et al., 1998).

**Structural chromosome abnormalities**

Approximately 2% of spontaneous miscarriages are associated with structural, as opposed to numerical, chromosome abnormalities. Structural abnormalities are re-arrangements of a portion of a chromosome, and include translocations, inversions, deletions and duplications (see Glossary). Translocations are the most common clinically important structural abnormality. In their balanced form they have no phenotypic effect. However, unbalanced translocations are likely to have an effect on the phenotype and the viability of the pregnancy.

An unbalanced structural abnormality ascertained in a miscarriage may well have caused the miscarriage, and could result from chromosomal segregation errors during gametogenesis in a parent with a balanced rearrangement. When products of conception contain an unbalanced structural rearrangement, it is customary to examine the parental chromosomes and determine whether a parent carries a balanced rearrangement. When products of conception contain an unbalanced structural rearrangement, it is customary to examine the parental chromosomes and determine whether a parent carries a balanced rearrangement which may put them at risk of having a further conceptus with additional and/or missing chromosome material (Jacobs et al., 1975).

The frequency of unbalanced gametes segregating during gametogenesis in an individual with a balanced translocation depends on the exact positioning of the translocation breakpoints. The great majority of balanced structural rearrangements appear to segregate in families and to be unrelated to any adverse reproductive outcomes (Jacobs et al., 1975; Morton et al., 1975). Hence a structural rearrangement in a couple presenting with repeated miscarriages may be co-incidental and not the cause of the miscarriage.

**Single-gene defects that cause infertility**

**Hypogonadotropic hypogonadism**

Normal secretion of pituitary gonadotropins depends on migration of gonadotropin-releasing hormone (GnRH) producing neurons from the olfactory placode to the forebrain and the development at puberty of pulsatile GnRH secretion from the hypothalamus into the portal circulation. Deficiency of this hypothalamic–pituitary system causes hypogonadotropic hypogonadism (HH), which occurs in approximately 1:8000 newborns in a 4:1 male:female ratio (Simoni and Nieschlag, 2007).

HH is the most likely type of female infertility to be associated with a specific genetic defect, usually affecting the hypothalamus rather than the pituitary. Kallmann syndrome is one genetic cause of primary amenorrhea which can be suspected on clinical grounds (anosmia) and for which there is a specific treatment (pulsatile GnRH administration). A specific genetic lesion can be identified in 50% of familial cases with isolated HH and in 30% of familial cases with Kallmann syndrome.

Of 315 patients with isolated HH, 6% had one or more affected relatives. Autosomal recessive inheritance was most likely but autosomal-dominant and X-linked recessive inheritance patterns also were observed (Bhagavath et al., 2006). All of the females had normal karyotypes and 30% had anosmia. Approximately 10% of the patients tested had mutations in the KAL1 gene or the gene for the GnRH receptor (GNRHR).

GnRH deficiency due to developmental defects of GnRH secreting neurons is usually associated with anosmia, constituting Kallmann syndrome. Several genes regulating GnRH synthesis, secretion or action have been identified, and systematic studies in humans and mice have confirmed their relevance for human reproduction (Table VI).

Inactivating mutations of the **KAL1** gene, encoding anosmin-1, which is an adhesion protein involved in synaptogenesis, have been identified in X-linked Kallmann syndrome. Patients with **KAL1** gene mutations may also present with bimanual synkinesia and renal anomalies. The **KAL2** gene encodes the fibroblast growth factor receptor 1 (FGFR1), a tyrosine kinase receptor involved in olfactory bulb development which interacts with anosmin-1. Inactivating **KAL2** gene mutations are found in autosomal-dominant Kallmann syndrome with hypogonadism, with or without anosmia (Dode et al., 2003; Pitteloud et al., 2006a). The phenotype of hypogonadism may vary considerably in these patients

### Table V. Chromosomal abnormalities and pregnancy loss: mechanisms of non-disjunction (chromosomes X, 16, 13, 18 and 21).

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* observed—infrequent maternal age; ** observed—frequent maternal age; *** observed—very frequent maternal age. N, normal; C, raised; A, abnormal recombination.
including complete hypogonadism without any pubertal development, partial pubertal development and delayed puberty with normal reproductive function (Pitteloud et al., 2006b). Additionally, associations with cleft palate or lip, tooth agenesis, ear anomalies and deafness have been described (Fig. 2). The KAL3 gene, which encodes the prokineticin receptor 2 (PKR2), a transmembrane heptahelical G-protein coupled receptor (GPCR), or its ligand prokineticin 2 (PK2), is involved in olfactory bulb development (Matsumoto et al., 2006). Inactivating mutations have been identified in patients with autosomal-recessive Kallmann syndrome (Dode et al., 2006). Among familial Kallmann syndrome cases, mutations in the KAL1, FGFR1 or PKR2/PK2 genes were detected with similar frequency. A further candidate relevant for Kallmann syndrome is the nasal embryonic GnRH factor (NELF) involved in GnRH and olfactory neuron outgrowth, for which a heterozygous deletion has been found in one patient with Kallmann syndrome so far (Pitteloud et al., 2007). Mutations within the chromodomain helicase DNA binding protein 7 (CHD7) gene have been identified in 58% of individuals with CHARGE association, a syndrome in which HH is associated with ocular coloboma, cardiovascular malformations, choanal atresia, growth retardation, anosmia and ear anomalies (Lalani et al., 2005).

Altered GnRH secretion may also be due to defective signalling through the kisspeptin GPCR54 (GPR54) system. Expressed at the surface of GnRH neurons, GPR54 induces GnRH secretion, while patients carrying inactive GPR54 variants exhibit abnormalities in gonadotropin axis activation (de Roux et al., 2003; Seminara et al., 2003). In contrast to secretion defects, resistance to GnRH is caused by inactivating mutations of the GNRHR, another GPCR expressed in membranes of pituitary gonadotropes. Inactivating GNRHR mutations have been found in ~40% of patients with isolated autosomal-recessive HH, while GPR54 mutations are less frequent (15%) (Simoni and Nieschlag, 2007). Individuals bearing inactivating GNRHR substitutions may present a wide phenotypic spectrum from partial to complete hypogonadism depending on the specific functional consequences of each mutation. Because the GNRHR is inactive in these GnRH resistant defects, the necessary treatment is gonadotrophins (Karges et al., 2003a, b).

The difficulty in predicting phenotype from genotype in individuals with HH suggests that phenotypic heterogeneity is due to digenic rather than monogenic inheritance (Pitteloud et al., 2007). In other words, the clinical variability in features associated with GnRH deficiency may reflect mutations of two distinct genes in an individual, which may generate a more severe phenotype than a single-gene alteration.
Premature ovarian failure

Premature ovarian failure (POF, OMIM 311360–300511) is a heterogeneous group of disorders with cessation of ovarian function before the age of 40 years and elevated gonadotrophins. POF occurs in one per 1000 women by age 30 years and in one per 100 women by age 40 years accounting for up to 10% of ovulatory female sterility (Coulam et al., 1986).

Mechanisms

POF may result from (i) a decreased pool of primordial follicles, (ii) an accelerated rate of follicular atresia or (iii) dysfunction in follicular recruitment or maturation. In most cases the aetiology is unknown, but POF can be associated with autoimmune disorders and systemic diseases like galactosemia, or it can be secondary to chemotherapy or radiotherapy. POF can also arise with X chromosome abnormalities, which is the indication to carry out a karyotype analysis in these patients.

Familial POF

Familial occurrence of POF and the high frequency of associated chromosomal abnormalities suggest a genetic component (Vegetti et al., 2000). The incidence of familial POF varies from 4 to 31% depending on the inclusion criteria adopted and whether the assessment of family history is detailed (Davis et al., 2000; Vegetti et al., 2000). Pedigree studies of affected families suggest that the inheritance of POF may be autosomal dominant sex-limited or X-linked with incomplete penetrance (Vegetti et al., 2000).

POF is associated with mutations in a small number of genes including INHA, which encodes inhibin alpha (Marozzi et al., 2002) and the gene for the FSH receptor (Fauser and Hsueh, 1995). Recent evidence in experimental and natural models documents the essential role of the paracrine effect of growth differentiation factors of transforming growth factor (TGFβ) superfamily (BMP15, GDF9) produced by the oocytes or granulosa cells in ovary development and progression of folliculogenesis (Fabre et al., 2006). In this regard, variants of these genes have been identified in women affected by POF, presenting variable phenotypes (Di Pasquale et al., 2006; Laissue et al., 2006; Kovanci et al., 2007). Furthermore, the observation that GDF9 gene variants are significantly more prevalent among mothers of dizygotic (DZ) twins than controls suggests that these gene are of particular relevance in the pathway regulating the ovarian follicle growth and maturation (Palmer et al., 2006).

Also, the forkhead box L2 (FOXL2) gene is responsible for the autosomal dominant, blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES, OMIM 110100), which may occur with POF (BPES type I) or without POF (BPES type II) but is rarely involved in idiopathic POF (Bodega et al., 2004). Also rarely involved are mutations in the eukaryotic translation initiation factor 2B genes (EIF2B) found in patients with ovarioleukodystrophy.

Fragile X premutation

The fragile X syndrome arising from an X-linked mutation of the fragile X mental retardation 1 gene (FMR1 gene) causes the most common form of familial mental retardation in men (Hundscheid et al., 2003). The FMR1 mutation involves expansion of a CGG triplet to more than 200 copies in the 5 prime untranslated region (5′ UTR) of the FMR1 gene (full mutation). The premutation involves 50 to 200 copies of the CGG triplet and is associated with tremor/ataxia in males (FXTAS) and POF in females (Hagerman and Hagerman, 2004). Production of FMR1 mRNA is decreased in the full mutation, but increased in the premutation (mRNA gain-of-function toxicity), which may underlie the altered ovarian function.

The incidence of POF among female premutation carriers is ~15–20% compared with 1% in the female population. Inversely, the frequency of the premutation allele in POF females is ~5–10% compared with <1% in the general population. The POF risk is 2-fold higher in women with the FMR1 premutation (Bodega et al., 2006; Ennis et al., 2006). Because women with POF are at increased risk of having an FMR1 premutation, they should be informed of the availability of fragile X testing and counselled about the implications for the family if the result is positive (Wittenberger et al., 2007).

X monosomy

Turner syndrome with X monosomy causes POF with primary amenorrhoea; mosaic forms may cause POF with secondary amenorrhoea. POF also presents with structural anomalies of the X chromosome such as terminal and interstitial deletion (Tonioło, 2006). Two loci (from Xq22 to Xq26 and from Xq27 to 28) appear to be critical for the POF phenotype. The mapping of different POF translocation breakpoints led to the identification of four interrupted genes, (DIAPH2 gene in proximal Xq22, XPNPEP2 gene in Xq25, DACH2 gene in Xq21.3 and POBF1 gene in Xq21.3). It remains unknown, however, whether these genes contribute to the aetiology of POF (Toniolo, 2006).

Thus, POF is a heterogeneous disorder that may have several genetic causes. Some genetic causes may be inherited as Mendelian factors; others seem to carry susceptibility variants that increase the risk of developing ovarian dysfunction.

Karyotype and FMR1 testing are indicated in most cases. Genetic counselling is essential for women who have POF or belong to families with POF.

Congenital absence of the uterus and vagina

Congenital absence of the uterus and vagina (Mayer–Rokitansky–Küster–Hauser) syndrome involves a failure of development of the Müllerian derivative of the vagina and the portions of the Müllerian ducts that are destined to become the uterus. The incidence is one per 4000 to 10 000 female births. Type I has complete aplasia of the vagina and uterus with two rudimentary horns and normal Fallopian tubes. Type II has vaginal aplasia with either symmetric uterine hypoplasia or aplasia of one horn, tubal malformation and other associated malformations.

A tendency to familial distribution suggests the potential for a genetic link, although few genes have been identified as causal. One candidate is mutation of the WNT4 gene, a developmental gene which regulates growth and differentiation during embryogenesis.

Anomalies of the Müllerian duct also have been described in association with maturity onset diabetes of the young (MODY) due to mutations of the TCF2/HNF1β gene (MODY5, OMIM 189907) (Fajans et al., 2001; Karges et al., 2007). Since discovery
of the Müllerian duct malformation may precede signs of diabetes, an oral glucose tolerance test should be performed in these individuals to detect this chronic metabolic disorder.

**Folate metabolism genes and fertility**

High homocysteine concentrations are associated with pregnancy loss and high concentrations of folic acid have been associated with an increase in twinning (Forges et al., 2007). The folate-methylation cycle demethylates homocysteine to produce methionine and then S-adenylmethionine (SAM), which is a prime source of methyl groups for DNA methylation and for the synthesis of nucleic acids, proteins and lipids. Deficiencies in the enzyme methylenetetrahydrofolate reductase (MTHFR), which is central to the methylation pathway, cause DNA hypomethylation and could be related to imprinting disorders (Dobson et al., 2007). The gene for MTHFR has two common polymorphisms (CC to TT at base pair 677 and AA to CC at base pair 1298) which have been studied in association with embryonic development and live birth after IVF. Among 602 women undergoing IVF, live birth was less likely (odds ratio, OR 0.24, 95% CI 0.08–0.71) with the homozygous 1298 CC variant of the MTHFR gene, than the AA variant; the C677T polymorphism had no effect (Haggerty et al., 2006). In a study of 197 couples, however, neither the C677T nor the A1298C variants were associated with embryo quality or pregnancy rate (Dobson et al., 2007). The conflicting results may reflect gene–gene as well as gene–environment interactions, which may be a source of bias in observational studies (Forges et al., 2007). Alternatively, the effects of the polymorphisms may be too small to have consistent effects in folate-replete populations or in women <35 years of age (Forges et al., 2007).

**Figure 3:** Developmental origin of PCOS (adapted from Abbott et al., 2002).

**Family and twin studies**

Familial clustering of PCOS cases has often been reported, suggesting a genetic origin of the disorder. In a Dutch twin study, concordance of PCOS was greater in monozygotic than in DZ twins (Vink et al., 2006). Hyperandrogenaemia, with or without menstrual cycle abnormalities, is common among sisters of PCOS patients. Familial hyperinsulinaemia is also common; elevated insulin levels were associated with hyperandrogenaemia, rather than with menstrual irregularity (Legro et al., 2002).

**Candidate genes**

A large number of case–control and family-based association studies have been performed to search for candidate genes in PCOS. The most obvious candidate genes to consider include (i) genes implicated in folliculogenesis (about which little is known), (ii) genes affecting insulin secretion or action or (iii) genes involved in the androgen biosynthetic pathway (Fig. 3). A plausible candidate gene in the androgen synthesis pathway is CYP11A, encoding the cholesterol side chain cleavage enzyme P450sc, because the increase in androgen production in PCOS appears to reflect a ‘global’ increase in steroid production by theca cells. A large case–control study found no association, however, between polymorphisms in CYP11A and either polycystic ovary morphology or testosterone levels (Gaasenbeek et al., 2004). Among nearly 50 other candidate genes studied, only one, so far, has been clearly associated with PCOS status (Nam Menke and Strauss, 2007; Urbanek, 2007). The one potential exception is a locus on chromosome 19p13.2, which is now thought to be in the region of the fibrillin 3 (FBNN3) gene (Stewart et al., 2006). Fibrillins are modulators of TGFβ proteins, but their potential role in PCOS remains to be determined. The main limitation of many of the published studies of potential candidate genes in PCOS is the lack of appropriate power (i.e. the populations studies are too small to either confirm or exclude candidate loci). This is important because, among the many potential candidate genes, the effect of any one gene is likely to be small.

While the search for candidate genes continues, genome-wide association studies of the kind that have been successfully applied in type 2 diabetes may be the most likely means to uncover the genes contributing to PCOS, even though these studies are expensive and require large populations (thousands) of cases and appropriate controls (Urbanek, 2007).
The genetics of endometriosis
A genetic aetiology of endometriosis was suggested many years ago with a report that first-degree relatives of women with severe endometriosis had a 6-fold higher risk than relatives of unaffected women (Simpson et al., 1980). Endometriosis appears to be inherited not as a Mendelian condition, but as a complex genetic trait influenced by environmental factors (Kennedy et al., 1995).

Family and twin studies
Clinical evidence of a genetic basis for endometriosis includes familial clustering in humans (Kennedy et al., 1995) and rhesus monkeys (Hadfield et al., 1997a); a founder effect in the Icelandic population (Stefansson et al., 2002); concordance in monozygotic twins (Moen, 1994; Hadfield et al., 1997b; Treloar et al., 1999a); a similar age at onset of symptoms in affected, non-twin sisters (Kennedy et al., 1996) and up to 15-fold higher prevalence in first-degree relatives of cases over the general population (Coxhead and Thomas, 1993; Moen and Magnus, 1993; Kennedy et al., 1998). Genetic traits may create a susceptibility to endometriosis although the disease emerges only after exposure to environmental risk factors (Giudice and Kao, 2004).

As in other diseases with complex genetic inheritance, there are difficulties in the interpretation of the clinical studies. The problems include a heterogeneous clinical profile (not all women with endometriosis have pain, infertility or a laparoscopy); ascertainment bias (relatives of women with endometriosis are more likely to ask for testing) and referral bias (only severe cases attend tertiary clinics where studies are done). Thus, the relative influence of genetic versus environmental factors remains uncertain.

Linkage and association studies
Plausible candidate genes for the aetiology of endometriosis, including detoxification and tumour suppressor genes, have emerged from linkage analysis and association studies (Giudice and Kao, 2004).

A definitive linkage analysis study is The International Endogene Study which analysed genetic variants in sister pairs and families (Kennedy et al., 1995, 2001; Treloar et al., 1999a, b, 2002). Among women that could be staged 2957 (54%) have minimal to mild disease and 2586 (46%) have more severe disease. A genome-wide scan of over 400 microsatellite markers identified one region of significant linkage (Maximum LOD score = 3.09) on chromosome 10q26 and one region of suggestive linkage (Maximum LOD score = 2.09) on chromosome 20p13 (Treloar et al., 2005). There is also suggestive evidence of one or more high-penetrance susceptibility loci with near-Mendelian inheritance on chromosome 7 in 248 of the families with ≥3 affected members (Zondervan et al., 2006). The parametric analysis produced a MOD score (equal to maximum LOD maximized over numerous possible inheritance models) of 3.30 at D7S484 for a recessive model with high penetrance. Critical recombinant analysis has narrowed the probable region of linkage down to overlapping 6.4 and 11 Mb intervals containing 48 and 96 genes, respectively.

The identification of endometriosis susceptibility genes should lead to a clearer understanding of the molecular and cellular mechanisms involved in the aetiology and pathogenesis of the disease and possibly lead to a categorization of the disease into logical sub-types based upon genetic information.

Genetic aspects of fibroids as a cause of female infertility
Uterine leiomyomas (fibroids) can be identified by ultrasound in 80% of African–American women and almost 70% of white American women by the time they reach menopause (Baird et al., 2003). Although fibroids theoretically may distort uterine anatomy, obstruct the tubal ostia or impede implantation, there is little evidence that fibroids cause infertility (Kolankaya and Arici, 2006). One exception is the observation among IVF patients that submucous fibroids impair success rates (Pritts, 2001). A genetic basis for fibroid presence is suggested by family and ethnic studies.

Family and ethnic studies
Family studies show that there is a 2.5-fold increased risk for fibroids among first-degree relatives of women with fibroids compared with relatives of unaffected women (Dixon et al., 2006). In ethnic studies, fibroids are three times more frequent in black compared with white women in America (Baird et al., 2003).

Hereditary leiomyomatosis renal cell carcinoma (HLRCC) is a familial cancer syndrome with an autosomal dominant hereditary pattern in which many patients develop uterine and cutaneous leiomyomas as well as renal cell carcinoma. The FH gene, which codes for the fumarate hydratase enzyme, contains mutations in ~90% of patients with HLRCC. This FH mutation may predict a predisposition in white but not black American women (Dixon et al., 2006).

Candidate gene studies
Analysis of candidate genes has not been successful. Fibroid growth appears to be estrogen-dependent, but microarray and candidate gene studies have not uniformly identified significant changes in genes for estrogen receptors, progestrone receptors or their nuclear receptor cofactors (Arslan et al., 2005).

Gene expression studies
Eight genes (ADH1, ATF3, CRABP2, CYR61, DPT, GRIA2, IGF2, MEST) were identified as over or under expressed by at least five of nine gene expression studies (Arslan et al., 2005). Genes regulating retinoid synthesis, IGF metabolism, TGF-β signalling and extracellular matrix formation have biological plausibility in the aetiology of fibroid development. ADH1 and CRABP2, for example, are involved in retinoid metabolism and also estrogen-dependent. It is also possible, however, that the differences in expression between fibroid and normal myometrial tissue were the result of the alpha error arising from the thousands of significance tests involved in these discovery studies.

Thus, family studies suggest that the development of fibroids may be genetically determined and candidate gene expression studies have identified over or under expression of some genes in fibroid tissue compared with normal myometrium. There remains, however, no conclusively proven genetic pathway for the development of fibroids and the disorder is so common that genetic studies are difficult to interpret.
Genetic aspects of RM

RM is the loss of three or more consecutive pregnancies, affecting 1% of couples trying to conceive (Rai and Regan, 2006). Among women with three or more previous spontaneous miscarriages, the risk of another one rises from 40% at age 35–39 years to 60% at age 40–44 years (Nybo Andersen et al., 2000) (Fig. 4). RM remains unexplained in up to 80% of cases after investigation, suggesting the possibility of genetic causes. Genetic counselling is needed by the 3% of partners that have an abnormal karyotype and in cases involving decisions about karyotyping the products of conception.

Genetic factors contributing to RM

Single-gene defects

Couples with RM are twice as likely as controls to have hereditary thrombophilias due to Factor V Leiden defect and prothrombin gene (G20210A) mutations (Kovalevsky et al., 2004). Since the population prevalence of each mutation is close to 5%, the prevalence in RM couples may be as high as 10%, and screening is indicated.

Levels of plasminogen activator inhibitor-1 (PAI-1) have been reported in some studies to be increased with RM (Sermon et al., 2007). These PGD outcomes are from collected national series in which RM was not necessarily the only clinical diagnosis (Franssen et al., 1994; Franssen et al., 2006; Stephenson and Sierra, 2006). While carriers of balanced translocations are normal, more than half of their gametes and embryos are unbalanced because chromosomal segregation is disrupted during meiosis. Thus it is prudent to arrange for peripheral blood karyotyping for couples with RM. If an abnormality is found, the couple should be referred to a clinical geneticist to decide on further family studies and to discuss the prognosis for a future pregnancy.

The prognosis in a future pregnancy is not desolate, however. In 73 pregnancies among 99 couples with a structural chromosomal abnormality, 33 (45%) ended in live birth (Carp et al., 2004). In 58 pregnancies among 51 similarly affected couples, 41 (71%) ended in live birth (Stephenson and Sierra, 2006). In 239 pregnancies among 278 couples with a structural chromosomal abnormality, 205 (83%) ended in live birth; the live birth rate was similar for reciprocal translocations, Robertsonian translocations and inversions (Franssen et al., 2006).

The C677T polymorphism in the MTHFR gene which controls DNA methylation was the subject of 26 case–control studies involving 2120 couples with unexplained RM and 2949 controls. In a meta-analysis there was a modest effect of the TT genotype on risk for unexplained RM (OR 1.49, 95% CI 1.12–2.00) compared with the CC genotype (Ren and Wang, 2006). The risk was higher in the five studies from China (OR 2.96, 95% CI 1.88–4.66), where the frequency of the MTHFR 677TT polymorphism was 25% in cases and 15% in controls, compared with 13 and 11% in cases and controls from other countries.

Recurrence risk

A miscarriage with a normal karyotype is more likely to recur than one with an abnormal karyotype, and the repeat miscarriage is more likely to have a normal karyotype (Lauritsen, 1976). Women with trisomy abortion, however, are 2.5-fold more likely to have another miscarriage with the same trisomy (homo-trisomy) (Warburton et al., 2004). When the chromosome involved in the two trisomy conceptions is not the same (hetero-trisomy), there may be an increased risk for non-specific nondisjunction, which is likely to be genetic in origin.

Recurrent pregnancy loss and structural chromosomal abnormality

One partner in ~3% of couples with RM has a balanced structural chromosomal abnormality (De Braekeleer and Dao, 1990; Clifford et al., 1994; Franssen et al., 2006; Stephenson and Sierra, 2006). While carriers of balanced translocations are normal, more than half of their gametes and embryos are unbalanced because chromosomal segregation is disrupted during meiosis. Thus it is prudent to arrange for peripheral blood karyotyping for couples with RM. If an abnormality is found, the couple should be referred to a clinical geneticist to decide on further family studies and to discuss the prognosis for a future pregnancy.

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The couple has a risk of an unbalanced translocation in a pregnancy ranging from 1 to 5%, indicating the need for prenatal diagnosis (Franssen et al., 2006). PGD is another option for translocation carriers. This requires the couple to undergo IVF to produce embryos for testing. In 469 PGD cycles among patients with translocations there were 123 clinical pregnancies (26% per PGD cycle, 35% per embryo transfer) (Verlinsky et al., 2004). In 282 PGD cycles for abnormal karyotype in which embryos were transferred, the clinical pregnancy rate was 25% per transfer (Sermon et al., 2007). These PGD outcomes are from collected series in which RM was not necessarily the only clinical diagnosis for couples with a translocation, but they are lower than the live birth rates from 45 to 83% to be expected with natural conception (Carp et al., 2004; Franssen et al., 2006; Stephenson and Sierra, 2006).
Karyotype testing in products of conception

The pattern of abnormalities found in products of conception from RM couples is not substantially different from the pattern in spontaneous losses shown in Table II, with the exception of fewer Turner syndromes in RM. Trisomy is the leading anomaly (31%, most often chromosomes 15, 16, 22 and 21), followed by polyploidy (9%), Turner syndrome (4%) and unbalanced translocations (2%).

Euploid products of conception would imply that there is a non-cytogenetic cause of RM, leading some authors to advocate karyotyping of all pregnancy losses in couples with RM (Stephenson et al., 2002). On the other hand, chromosome abnormalities are so common that a euploid report is far from reassuring about a future pregnancy. Given the overall favourable prognosis for idiopathic RM and the cost of karyotyping products of conception, it seems reasonable to limit the procedure to couples undergoing a specific treatment for RM: a normal karyotype in a failed pregnancy would suggest the loss was due to treatment failure rather than aneuploidy.

PGS for unexplained RM

Couples with unexplained RM may be at higher than average risk for embryo aneuploidy compared with age-matched controls. In one series of 241 PGS cycles in RM couples with FISH testing for chromosomes 13, 16, 18, 21, 22, X and Y, the pregnancy rate was 37% per transfer (Rubio et al., 2005). In another series of 69 cycles there were 10 pregnancies (15%) (Platteau et al., 2005). The ESHRE PGD Consortium guidelines state that similar couples have a high chance of conceiving naturally (Thornhill et al., 2005). Two studies support that statement: of 325 couples with idiopathic RM, 70% (n = 226) conceived, with a 75% success rate (Brigham et al., 1999); and among 201 women with three or more consecutive first trimester miscarriages and normal investigations, the live birth rate in the next pregnancy was 69% (Clifford et al., 1997). In couples with unexplained RM, similar to those with a known translocation, the live birth rate with natural conception is higher than any success rate reported after preimplantation diagnostic procedures.

Conclusions

The clinical practice of reproductive medicine is increasingly dependent on methods for genetic diagnosis to identify the correct management. Chromosomal defects explain a high proportion of human pregnancy failures. Nevertheless, karyotyping tests only at the most superficial level of genetic abnormality and testing in the future should be developed to detect other potential genetic defects which may be clinically important.

Single-gene defects are rare causes of infertility, but in clinical presentations such as HH single-gene defects may be found in a high proportion of the cases.

Karyotype and FMR1 testing are indicated in most POF cases and genetic counselling is essential for women who have POF or belong to POF families.

Complex genetic inheritance patterns may explain the small tendency to a familial distribution found in common disorders such as PCOS and endometriosis. Genetic research methods are likely to improve the understanding of what are now thought to be cases of unexplained infertility and unexplained RM. Couples with infertility or RM who have a single-gene defect or a translocation should have professional genetic counselling.

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Glossary

(Definitions not referenced are from Stedmans Online Medical Dictionary).

Genetics terms

Acrocentric: a chromosome which has the centromere close to one end as in chromosomes 13–15 and 21–22.

Aneuploidy: the occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement, or, any chromosome number that is not an exact multiple of the haploid number. http://ghr.nlm.nih.gov/ghr/glossary/aneuploidy.


Mosaic: the occurrence of two or more cell lines with different genetic or chromosomal constitutions within a single individual or tissue. The result is an organism or one of its parts that is composed of cells of more than one genotype. http://ghr.nlm.nih.gov/ghr/glossary/mosaic.

Non-disjunction: failure of one or more pairs of chromosomes to separate at the meiotic stage of karyokinesis, with the result that both chromosomes are carried to one daughter cell and none to the other.

Polyploidy: the cell nucleus contains three or more haploid sets. Cells containing three or four multiples are referred to as triploid or tetraploid.

Polysomy: a specific chromosome is represented more than twice. Cells containing three or four homologous chromosomes display trisomy or tetrasomy.

Single-nucleotide polymorphism: or SNP (pronounced ‘snip’), is a small genetic change, or variation, that can occur within the DNA sequence. The genetic code is specified by the four nucleotide ‘letters’ A (adenine), C (cytosine), T (thymine) and G (guanine). SNP variation occurs when a single nucleotide, such as an A, replaces one of the other three nucleotide letters—C, G, or T. http://www.ncbi.nlm.nih.gov/About/primersnps.html.

Types of chromosome abnormalities

Numerical chromosomal abnormalities: a number of chromosomes in a somatic cell other than two, such as monosomy or trisomy, or more than a duplicate set of chromosomes (triploidy, tetraploidy).

Inversion: an inversion involves the breakage of a chromosome in two places; the resulting piece of DNA is reversed and re-inserted into the chromosome. Genetic material may or may not be lost as a result of the chromosome breaks. An inversion that involves the chromosomes’ constriction point (centromere) is called a pericentric inversion. An inversion that occurs in the long (q) arm or short (p) arm and does not involve the centromere is called a paracentric inversion.

Deletion: deletions occur when a chromosome breaks and some genetic material is lost. Deletions can be large or small, and can occur anywhere along a chromosome.

Duplication: duplications occur when part of a chromosome is copied (duplicated) too many times. This type of chromosomal change results in extra copies of genetic material from the duplicated segment.

Translocation: a translocation occurs when a piece of one chromosome breaks off and attaches to another chromosome. This type of rearrangement is described as balanced if no genetic material is gained or lost in the cell. If there is a gain or loss of genetic material, the translocation is described as unbalanced. Translocation may be reciprocal or Robertsonian.

Reciprocal translocation involves exchange of segments from two different chromosomes.

Robertsonian translocation involves the acrocentric chromosomes 13, 14, 15, 21 and 22, where the entire long arm attaches to another chromosome at the centromere and the short arms of both are lost.

Investigation methods

Comparative genomic hybridization (CGH): a molecular cytogenetic technique that allows detection of DNA sequence copy number changes throughout the genome in a single hybridization. CGH is based on co-hybridization of differentially labeled tumor and normal DNA to human metaphase chromosomes. Also called chromosomal microarray analysis (CMA). http://cgap.mfnih.gov/Protocols/DNARNAProteomicAnalysis/DNA/CGH.html.

Cytogenetics: the study of chromosomes, their structure and their inheritance.

Fluorescence in-situ hybridization (FISH); a process which paints chromosomes or portions of chromosomes with fluorescent molecules. This technique is useful for identifying chromosomal abnormalities and gene mapping. http://www.genome.gov/glossary.cfm?key=fluorescence%20in%20situ%20hybridization%20(FISH).

Genome-wide association study: a study design that involves genotyping cases and controls at a large number (up to 500,000) of SNP markers spread (in some unspecified way) throughout the genome to look for associations between the genotypes at each locus and disease status. www.stats.ox.ac.uk.

LOD score: a likelihood ratio statistic which estimates whether two loci are likely to lie near each other on a chromosome and are therefore likely to be inherited together. An LOD score of three or more is generally taken to indicate that the two loci are close. http://www.genome.gov/glossary.cfm?key=LOD%20score.

Polymerase chain reaction: a powerful method for amplifying specific DNA segments which exploits certain features of DNA replication. Replication requires a primer and specificity is determined by the sequence and size of the primer. The method amplifies specific DNA segments by cycles of template denaturation; primer addition; primer annealing and replication using thermostable DNA polymerase. http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-P/polymerase_chain_reaction.html.

ESHRE Capri Workshop Group

A meeting was organized by ESHRE to discuss the above subjects. The speakers included: J. Collins (McMaster University, Hamilton, Canada), K. Diedrich (Klinik für Frauenheilkunde und Geburtshilfe, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Germany), S. Franks (Institute of Reproductive and Developmental Biology, Imperial College London, Hammersmith Hospital, London, UK), J.P.M. Geraedts (Head, Department of Genetics and Cell Biology, University Maastricht, The Netherlands), P.A. Jacobs (Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK), B. Karges (University Children’s Hospital, Pediatric Endocrinology and Diabetes, University of Ulm, Germany), S. Kennedy (Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, UK), A. Marozzi (Department of Biology and Genetics for Medical Sciences, University of Milano, Italy), L. Regan (Division Surgery, Oncology, Reproductive Biology, Anaesthetics (SORA), Department of Obstetrics and Gynaecology, Imperial College at St Mary’s Hospital Campus, London, UK). The discussants included: D.T. Baird (Centre for Reproductive Biology, University of Edinburgh, UK), P.G. Crosignani (II Department of Obstetrics and Gynecology, University of Milano, Italy), P. Devroey (Centre for Reproductive Medicine, Universitair Ziekenhuis Vrije Universiteit Brussel, Belgium), E. Diezfalusy (Karolinska Institutet, Stockholm, Sweden), J.L.H. Evers (Department of Obstetrics and Gynecology, Academic Hospital Maastricht, The Netherlands), B.C.J.M. Fauser (Department of Reproductive Medicine and Gynecology, University Medical Center, Utrecht, The Netherlands), L. Fraser (Reproduction and Rhythms Group, School of Biomedical and Health Sciences, Kings College London, UK), L. Gianaroli (S.I.S.Me.R., Bologna, Italy), A. Glisier (Family Planning and WW Services, Edinburgh, UK), I. Liebaers (Centre for Medical Genetics, Universitair Ziekenhuis, Vrije Universiteit Brussel), G. Ragni (U.O.C. Sterilità di Coppia ed Andrologia, Fondazione Policlinico, Mangiagalli e Regina Elena, Milano, Italy), A. Sunde (Department of Obstetrics and Gynecology, University of Ulm, Germany), S. Kennedy (Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, UK). The report was prepared by J. Collins (Hamilton) and P.G. Crosignani (Milano).

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