Preimplantation Genetic Diagnosis

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Foreword

Few developments are anticipated to affect human beings more profoundly in the coming years than the knowledge gained from the complete sequencing of the human genome. This knowledge combined with the power and sensitivity of recombinant DNA technologies promises to revolutionize the diagnostic, predictive and life-enhancing capabilities of medicine. Nowhere will these medical developments become more apparent than in the field of preimplantation genetics, particularly as the functional nature of each of the thousands of human genes is defined.

More than three decades have now passed since the first deliberate attempt to perform preimplantation diagnosis of genetic disease by Robert G. Edwards and colleagues: the chromosome sex of rabbit blastocytes was successfully determined by identifying the sex chromatin body in trophoblast cells at approximately day 5 following fertilization. It was not until the advent of human in vitro fertilization in the 1980s, however, that the possibility of diagnosing genetic disease in the preimplantation embryo could begin to be realized. The integration of three technical advances at the time is credited with establishing a practical basis for the field of preimplantation genetic diagnosis. These were (1) methods of in vitro fertilization (IVF) of human oocytes that were rapidly adopted and practiced worldwide; (2) the development of DNA technologies to such a level that single cells could be subjected to a series of genetic analyses; and (3) the introduction of micromanipulation devices that made possible highly efficient and successful excision of single cells from preimplantation embryos. With the 1990 report by Alan Handyside and Robert Winston on the birth of healthy females after preimplantation testing for sex, preimplantation human genetics and diagnosis became a reality. Although only ten years have passed, remarkable advances in technology and in the science of preimplantation genetics have transpired.

Genetic analyses performed on human preimplantation embryos have provided valuable information on both technical and biological factors. To name just a few: (1) the problem of allele dropout (ADO), whereby preferential PCR amplification of one allele, particularly in cases of compound heterozygotes, has resulted in diagnostic errors; (2) the introduction of fluorescence in situ hybridization (FISH) for up to seven, clinically significant chromosomes, which when applied to women of advanced maternal age, may result in enhanced implantation and pregnancy rates; (3) the high level of chromosome mosaicism in preimplantation embryos, which appears to arise as a post-zygotic event and, in certain cases, may account for fertility and/or high rate of recurrent spontaneous abortion; and (4) the initial definition of the expression of genes unique to the preimplantation period. The use of ICSI (intracytoplasic sperm injection), as an extension of micromanipulation technology, provides the means to enhance fertilization rates as well as to circumvent a number of factors responsible for male infertility.

Much of the workings and implications of preimplantation genetics and diagnosis have not been without controversy and differences between investigators specializing in preimplantation genetic diagnosis exist. While allele dropout continues to be a problem, laboratory approaches have been devised to monitor its occurrence and frequency. The utility of FISH in routine IVF cycles has not been resolved through randomized trials. Studies are in progress to determine whether failure of expression of genes acting as cell cycle checkpoints accounts for the apparently haphazard chromosome distribution in preimplantation embryos and whether genetic analysis should preferentially be performed on polar bodies or blastomeres. Initial concerns about the effects of ICSI on the conceptus, particularly mental development and fertility, may not be warranted. Nevertheless, there has been a call to karyotype all males where ICSI is to be applied, in order to prevent transmission of a chromosome aberration.

This book represents the most recent advances and developments in the field of preimplantation diagnosis and genetics. The individual chapters are authored by leading authorities in the field, each having made seminal contributions to our understanding of the biology and genetics of preimplantation embryos. The editors, Joyce Harper, Alan Handyside and Joy Delhanty, internationally recognized for their contributions to the field of preimplantation genetics and diagnosis, have used their collective wisdom, knowledge and experiences to provide the reader with a complete overview of this field. In every sense, this book provides a view of the past, present and future status of preimplantation genetics and diagnosis, from genetic counselling to micromanipulation of single cells to sophisticated methods of DNA analysis. Not only does the text detail the requirements for establishing a quality program in preimplantation genetics and diagnosis but to its credit, sets the stage for critically addressing and resolving a myriad of biological questions and ethical concerns involving the use and application of human embryos in preimplantation genetic and diagnosis studies.

Eugene Pergament

1 Introduction

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HISTORY OF PGD

In the last 20 years the areas of in vitro fertilization (IVF) and genetic testing have become a major part of treatment for couples trying to obtain a healthy family. IVF clinics are now found in every corner of the world, and many thousands of babies have been born. Preimplantation genetic diagnosis (PGD) is a marriage of IVF and prenatal diagnosis. For the last 10 years it has been possible to carry out genetic testing in the preimplantation embryo, so that a pregnancy can be started knowing that the embryo is free from a particular disease.

It was the team at the Hammersmith Hospital IVF Unit in London who set about performing the first cases of PGD in the late 1980s. As well as the technical problems that PGD presented, the Human Fertilisation and Embryology Act was going through the British parliament at this time and threatened to ban all embryo research in the UK, which would have blocked any further work leading to the development of PGD. Along with the group Progress, many people in the UK presented a logical and ethical debate concerning the usefulness of embryo research and the first PGD babies were conceived just as the Human Fertilisation and Embryology Act was passed, which finally permitted embryo research under specific conditions including research into the diagnosis of inherited diseases.

PGD involves two stages: IVF/embryo biopsy and genetic testing. In 1988, the Hammersmith team reported that the biopsy of up to two cells from the eight cell stage embryo did not affect the development of the embryo to the blastocyst stage, or embryo metabolism (Hardy *et al.*, 1990). The diagnoses, however, did not prove so easy to develop. Genetic testing has always relied on numerous cells to perform a reliable test, but with PGD only two cells were available and so very sensitive tests were required. The first single cell diagnostic test used the polymerase chain reaction (PCR) to amplified a Y chromosome repeat sequence to sex embryos for patients carrying X-linked disease (Handyside *et al.*, 1990). This was not an ideal genetic test as embryos showing no result were assumed to be female and such embryos were considered for transfer. Any test which relies on a negative result should not be used in a diagnostic situation. Unfortunately a diagnostic error occurred in one of the first seven pregnancies and the pregnancy was terminated as no specific molecular test was available for this disease (Handyside *et al.*, 1990). This misdiagnosis may have been due to amplification failure, an anucleate cell (or failure to put the cell in the tube) or mosaicism where an XO cell was biopsied from an XY embryo. All of these phenomena will be discussed in later chapters and summarized in Chapter 14.

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At the same time, the Chicago group published a different approach to PGD, that of polar body biopsy (Verlinksy *et al.*, 1996).

The Hammersmith team knew that their genetic test was not ideal and so they joined forces with the Genetics Department at University College London. UCL had been working on fluorescence in situ hybridization (FISH) and used this technique in human embryos as a method of determining the presence of the X and Y chromosome for embryo sexing (Griffin *et al.*, 1991, 1994). Also at this time, two groups in the USA, at Cornell and Chicago, started successful PGD programmes.

Now there are at least 40 centres in 17 countries offering or developing PGD (Verlinsky & Kuliev, 1998). However, the development of the technique has been fraught with problems such as contamination, allele dropout and chromosomal mosaicism, making the PGD procedure more complicated than was originally thought. But PGD is a successful procedure and has helped and will continue to help many hundreds of couples around the world.

Since PGD is such a complicated procedure, this book has been written to try to cover all the important principles involved in PGD. It is designed for the IVF and genetics team who may be interested in finding out more about PGD. Therefore, sections on IVF are included to help the geneticists and on genetics to help the embryologists. Details of the procedures used, clinical aspects of PGD, ethics and the law, and finally the future of PGD are also covered. This introduction outlines some of the basic principles that apply to PGD.

GENETIC DISEASE

The detection of genetic disease in the human embryo before implantation gives parents the chance of starting a pregnancy knowing that the baby will be free of the inherited disorder that is prevalent in their family.

Genetic disease can be transmitted in a number of different ways and these are detailed in the chapter on the inheritance of genetic disease. Autosomal recessive inheritance requires that both genes have a mutation, so that a carrier has one normal and one mutated gene. Therefore, if both partners are carriers, there is a one in four chance that they will have a child with that disease. Examples of autosomal recessive diseases are cystic fibrosis and β-thalassaemia. Autosomal dominant diseases only require one copy of the mutant gene for the person to be affected, and so only one parent needs to have an abnormal gene for the disease to be passed on to their offspring. With this type of inheritance 50% of their offspring will be affected. Inherited cancer predisposition, Marfan's syndrome and Huntington's disease are examples of dominant disorders.

X-linked recessive conditions involve a gene carried on the X chromosome and only affect males. The mother is a carrier, and she is at 50% risk of passing on the abnormal X chromosome to her sons. Her daughters are at 50% risk of being carriers. There are over 400 X-linked diseases classified and for many the exact genetics has not been established.

A recently identified class of genetic diseases is the triplet repeat disorders which are caused by an expansion of a triplet repeat within a gene, causing the gene to be abnormal. These are an interesting group of diseases as the severity of the disease

often depends on the number of repeats. Fragile X syndrome and myotonic dystrophy are triplet repeat disorders. Fragile X carriers have an additional problem in that they may experience premature menopause.

An important group of disorders is caused by chromosome abnormalities. They usually manifest themselves as translocations, where two chromosomes have broken and rejoined incorrectly. Patients carrying a balanced translocation are usually phenotypically normal, as all the genetic information is present, but when they come to reproduce, their gametes may have an unbalanced genotype and this leads to a chromosomally unbalanced embryo. Most chromosome abnormalities are not compatible with life and so these patients experience repeated miscarriages or infertility. As well as translocations, patients may carry insertions, inversions or other rearrangements.

Any woman who becomes pregnant is at risk of having a child with a chromosome abnormality due to aneuploidy. This is where the chromosomes in the oocyte undergo meiosis incorrectly and so an extra or missing chromosome is present in the oocyte. This will lead to a chromosomally abnormal embryo, which in most circumstances will spontaneously abort (an extra copy of chromosome 16 is commonly seen in spontaneous miscarriages). It occurs most commonly for chromosomes 13, 16, 18, 21, 22 and the sex chromosomes. The risk of aneuploidy increases with age, but it can happen to a woman of any age. Therefore, methods have been developed to screen all pregnant women to detect which pregnancies may be carrying a chromosomally abnormal child.

PRENATAL DIAGNOSIS

Methods of screening and prenatal diagnosis are discussed in detail in Chapter 3. Briefly, screening can be achieved by examining markers in the maternal serum in the first or second trimester (serum screening) or by measuring the nuchal translucency in the first trimester (ultrasound screening). These methods together, also taking age into account, can pick up approximately 90% of chromosomally abnormal pregnancies. Women found to have a positive screen are offered prenatal diagnosis to determine whether their pregnancy is chromosomally normal.

For couples who have a positive screening test or who are known to be at risk of transmitting a genetic disease, there are two methods currently used for prenatal diagnosis: chorionic villus sampling (CVS) and amniocentesis. CVS is usually used for couples who are known to be at a specific risk as it can be performed early in the pregnancy. A small piece of the placenta is removed by a transabdominal or transvaginal route and can be used for genetic testing and chromosome analysis. Amniocentesis is performed in the second trimester and is often used when the result of screening shows the pregnancy may be at risk. About 15 ml of amniotic fluid is aspirated from the amniotic sac and can be used for molecular genetic or chromosome analysis.

Once the prenatal diagnosis sample has been taken, genetic or chromosome analysis is performed. Genetic testing is usually performed using PCR to see if the fetus is carrying the normal or abnormal gene. To examine the chromosomes a karyotype is performed where the cells are cultured under special conditions to

arrest the cells in metaphase and elongate the chromosomes. The chromosomes are stained with Giemsa (G-banding), which gives a specific banding pattern to each chromosome. As well as being a count of the chromosomes present, translocations, rearrangements and insertions can be detected.

GENETIC COUNSELLING

The aim of clinical genetics is 'To enable people and families with a genetic disadvantage to live and reproduce as normally as possible'. The first stages are diagnostic confirmation, establishment of a family history and counselling to discuss the implications. Before any couple opts for PGD they must be aware of the alternatives. Prenatal diagnosis is an easier option technically and has been performed in many thousands of cases around the world. However, some couples do not wish to consider terminating an affected pregnancy or they may miscarry before prenatal diagnosis can be offered. Therefore, any couple who have been diagnosed as carrying a genetic disease need to see a genetic counsellor to discuss the implications of the disease they are carrying, the risk factor and the alternatives for them to try and achieve a healthy family. In the UK, the majority of patients see a genetic counsellor before they are referred to the PGD centre, and so they are aware of the alternatives. The genetic counsellor is seen on an 'open access' basis which allows further discussion and counselling if required. Patients interested in PGD need to then see the PGD team to discuss what is involved in PGD, the feasibility, problems that may be encountered, limitations of the procedure, likelihood of misdiagnosis, pregnancy rates and the risk of multiple pregnancy. The patients have to arrive at their own informed choice. However, factors such as funding for treatment or availability of treatment may influence this choice.

IVF

For PGD the patients need to undergo IVF, even though in some circumstances they are fertile, as embryos need to be generated in vitro. IVF is a well-established procedure and the common methods used are covered in Chapter 5. Before a patient embarks on IVF several checks are performed in both partners. The sperm count is analysed and the male may undergo a physical examination. In the female, a gynaecological examination is undertaken, the uterus is checked and several hormone tests may be performed. Once all these procedures are carried out, an IVF cycle may commence. The woman is downregulated to ensure that her normal menstrual cycle is shut down. An exogenous source of follicle-stimulating hormone (FSH) is administered to stimulate oocyte growth. More recently this has been provided in the form of recombinant FSH. The IVF cycle is monitored closely using ultrasound and blood tests to measure the oestrogen levels and at a critical time, an injection of human chorionic gonadatrophin (HCG) is administered to stimulate the final stages of oocyte maturation. Approximately 36 hours later the oocytes are collected, usually by ultrasound guided aspiration, and placed in culture. During

this time the sperm is prepared, usually using a density gradient to separate live, motile sperm from dead sperm, cells and debris that are found in the ejaculate. The day following insemination, day 1, fertilization should have occurred and a zygote observed (see Chapter 6; p. 91). On day 2 post insemination, the zygote should have cleaved to two to four cells and the following day to approximately eight cells. In IVF treatment cycles the embryos are usually transferred on day 2 or 3.

With the advent of IVF, several groups tried to develop methods of overcoming male infertility. Two methods were initially developed: partial zona dissection or PZD and subzonal sperm insemination (SUZI). Both techniques reported success (see Chapter 5; p. 53). However, in the early 1990s these methods were replaced by ICSI (intracytoplasmic sperm injection), which has become the most important advance in IVF and is now performed worldwide.

It has been found that human embryos exhibit cleavage stage arrest, such that less than 50% of embryos reach the blastocyst stage in vitro. This is mainly due to insufficient culture conditions as the requirements of the embryo change from fertilisation to the blastocyst stage. Recently sequential culture medium has been developed and this has led to a trend to transfer blastocysts for certain IVF patients, even though the rate of embryos reaching the blastocyst stage is still low.

Overall the pregnancy rates in IVF have remained at around a 20% delivery rate per cycle for more than 20 years. In the UK the Human Fertilisation and Embryology Authority (HFEA), which was set up as a result of the Human Fertilisation and Embryology Act, collect detailed information on each IVF cycle conducted in the UK. These official figures have shown a disappointingly low IVF pregnancy rate. This has been one of the rate-limiting steps of PGD, as the PGD pregnancy rate has also been reported to be around 20% (ESHRE PGD Consortium, 1999, 2000).

PREIMPLANTATION EMBRYO DEVELOPMENT AND GENETICS

Before undertaking PGD or developing new techniques it is important to understand preimplantation embryo development and genetics (see Chapters 6 and 7).

There are many areas of preimplantation embryo development that can influence PGD. For PGD cells have been removed in the form of polar bodies, blastomeres or trophectoderm. Polar bodies are essentially waste products of meiosis, and so it is assumed that they are not required for further development. It has been shown that the loss of one or two blastomeres from a 6–10-cell embryo does not affect further development. However, it is at this stage that the embryo undergoes compaction, forming gap and other junctions to allow intercellular communication. These calcium-dependent junctions can hinder embryo biopsy, as it can be difficult to separate the cells. Blastocyst biopsy has been proposed, but even with today's culture conditions, the majority of embryos arrest in culture.

One of the most important factors that has come to light in recent years is that a single cell taken from an embryo may not be representative of the rest of the embryo. Using FISH to analyse all the cells from an embryo, high levels of chromosomal mosaicism are found to exist. Four groups of chromosome patterns have been described (see Chapter 7; p. 110). Of all the chromosome arrangements

observed, mosaicism may cause problems for PGD. It is probable that chromosome mosaicism at these stages is a normal part of development and the abnormal cells do not go on to form the embryo proper (if they did the fetus would probably abort).

The significance of mosaicism for PGD is discussed in detail in the section on misdiagnosis in Chapter 14 (p. 241).

CLINICAL ASPECTS OF PGD

During consultations to discuss PGD a large amount of information is discussed with the couple and so it is advisable to give the patients information leaflets on IVF and PGD and a written summary of the consultation. Communication with the patient's GP and clinical geneticist is essential. After the consultation the patients should be given time to discuss the option of PGD, but if they decide to embark on treatment, all the necessary preliminary tests required for IVF should be undertaken (see above and Chapter 5).

It is important to explain to the patients exactly what will occur during the PGD cycle; which diseases are being tested for, the limitations of the procedure, the possibility that all of the embryos may be affected, and the pregnancy rates must be discussed with the patients before treatment. For PGD to be successful, a good number of embryos are required so that at least two unaffected embryos of good morphology are available for transfer. Therefore, patients require quite aggressive stimulation.

For PGD, ICSI is used when a PCR diagnosis is performed to avoid contamination by sperm that may become embedded in the zona during in vitro fertilization.

EMBRYO BIOPSY

IVF theoretically allows several approaches to genetic diagnosis at the preimplantation stage. These are polar body analysis, biopsy at the cleavage stage or blastocyst stage biopsy. The majority of clinics offering PGD are currently performing cleavage stage biopsy.

There are two stages to cleavage stage embryo biopsy: zona drilling and blastomere aspiration. A micromanipulator is used coupled to an inverted microscope. This equipment is now fairly common in IVF clinics as it is used for ICSI. Micropipettes are used to manipulate the embryo. The embryo is immobilized using gentle suction and a hole is drilled in the zona and blastomeres are aspirated from the embryo. The procedure is not technically difficult, but problems arise due to compaction of the embryo at the eight-cell stage (see above and Chapter 6). It is rare to destroy the embryo totally, but more common is the lysis of the biopsied cell. Using $Ca^{2+}Mg^{2+}$-free medium to perform the embryo biopsy can reduce this. If a viable blastomere is not retrieved from the embryo then there will be no genetic material to perform the diagnosis and so the embryo cannot be considered for transfer. Recent data from the ESHRE PGD consortium (1999; see also Chapter 4) shows that the biopsy is successful in 97% of cases.

Polar body biopsy was developed by the Chicago group and has the advantage that the material taken from the oocyte and zygote is a waste product of meiosis. However, the technique is limited as only maternal genes and chromosomes can be examined and also a two-step procedure may be required, as the second polar body is not extruded until after fertilization but the first polar body degenerates rapidly.

In the past blastocyst biopsy had never been an option as most embryos arrested at the cleavage stage. This technique would give more cells for the diagnosis, but would also result in fewer embryos, as reports claim 50–70% of embryos from selected groups of patients reach the blastocyst stage. Therefore on occasions there would be few or no blastocysts for biopsy and so the chance of a successful PGD cycle would be reduced.

PCR

There are two methods that have been used to perform the diagnosis on the biopsied cells: PCR and FISH. PCR is a molecular technique that is used for the diagnosis of single gene defects, dominant disorders, sexing for X-linked disease and the diagnoses of triplet repeat disorders. The development of new diagnoses has been slow as single cell PCR has encountered some problems, namely contamination and preferential amplification or allele dropout (ADO). Preferential amplification or ADO occurs when one allele is preferentially amplified over another. If both partners are carrying the same mutation, this will not lead to the misdiagnosis of an affected embryo. However, for dominant disorders if the mutated allele is not amplified from an affected embryo, the embryo will be misdiagnosed as normal. Therefore it is vital that PCR protocols ensure that preferential amplification and contamination are eliminated from the PCR procedure.

Techniques to amplify and analyse DNA from a single cell have improved in recent years. Methods such as restriction endonuclease digestion, heteroduplex analysis, single strand conformational polymorphism, denaturant gradient gel electrophoresis and fluorescent PCR have been used. Additionally the use of short tandem repeats (STRs) can be used to ensure the DNA amplified is embryonic in origin and linked markers can be used when the mutation is unknown.

Methods have been employed to overcome the limitation of single cell analysis such as multiplex PCR and whole genome amplification. By using whole genome amplification there is sufficient DNA to perform many PCR reactions, and techniques such as comparative genomic hybridization (CGH) (see Chapter 14).

FISH

Karyotyping from a single embryonic nucleus is problematic and so FISH has been used to examine chromosomes from preimplantation embryos for PGD. Originally FISH was used to sex embryos for couples at risk of transmitting X-linked disease, using probes for the X and Y chromosome, but more recently it has been used for

couples carrying chromosome abnormalities such as translocations or for older women by screening for aneuploidy.

For couples at risk of passing on an X-linked disorder for which there is, as yet, no specific molecular diagnosis the current treatment offered is fetal sex determination and selective abortion of all males, of which half will be unaffected. There are also couples who have had to suffer repeated terminations of pregnancy, with or without a specific diagnosis. Therefore, PGD of embryo sex provides an alternative approach for these couples.

The group of patients who are referred most commonly for PGD are those who are carrying a chromosomal abnormality, usually a translocation. This group are often referred for PGD because they may experience infertility (men referred for ICSI have a higher chance of carrying a chromosome abnormality) or they often experience repeated miscarriages due to a chromosome imbalance in the embryo. This group of patients are probably the most committed to PGD, as they often do not get to the stage of prenatal diagnosis and so may have no alternative.

ETHICS AND THE LAW

PGD has attracted a large amount of media attention and as a result in most countries it is more regulated than prenatal diagnosis. The problem encountered with PGD is the worry that the embryo can be chosen or manipulated before implantation and concern that Aldous Huxley's 'Brave New World' is getting nearer. This idea of 'designer babies' has resulted in PGD being banned in some countries, and highly regulated in others. In some countries, prenatal diagnosis and the abortion of a second trimester pregnancy seems more acceptable than the diagnosis at cleavage stages before implantation.

Since the diagnosis is performed before the pregnancy, PGD may be used for the treatment of some diseases in a different manner to prenatal diagnosis. For example, for patients carrying an X-linked disease, the couple may opt not to transfer carrier females, so that the disease is eliminated from their family. PGD may also be more acceptable for couples at risk of transmitting late onset disorders, such as inherited cancers. It has also been suggested that PGD could be used to ensure that a sibling is a suitable match for an already affected child that needs a suitable organ donor or for sex selection for social reasons. Some of these options, especially the last two, may be ethically unacceptable for some people, and countries have to decide what they find acceptable.

THE FUTURE

Of most concern in PGD have been the reported misdiagnoses. To date there have been two for cystic fibrosis, and one report each for sexing, myotonic dystrophy, β-thalassaemia and trisomy 21. Preferential amplification contamination or chromosomal mosaicism may have caused the misdiagnoses.

Essentially, mosaicism does not pose a problem for the PGD of sex, or autosomal recessive diseases if both partners carry the same mutation. For heterozygous

embryos, or dominant disorders, mosaicism could be a problem, but DNA markers for the chromosomes can establish how many copies of each chromosome are present and where they are from (maternal, paternal or contamination). Mosaicism causes most problems for the PGD of chromosome abnormalities and aneuploidy. Therefore patients should be made aware of this phenomenon and that there may be a slight chance of a misdiagnosis. This would be reduced if two cells are biopsied from the embryo, but this is not always technically possible.

Allele dropout or preferential amplification is a technical problem that can be solved with efficient PCR techniques and work-up. Techniques such as multiplex PCR and whole genome amplification can be used to gain more information from a single cell. Multiplex PCR can be used to ensure ADO does not interfere with the diagnosis. As well as amplifying the area of the mutation, a second fragment containing a linked polymorphism can simultaneously be amplified. The chance of both sites experiencing ADO is low.

For the diagnosis of chromosome abnormalities, almost every patient that is referred has different chromosomes involved and so working up a diagnosis for such couples has proved time-consuming and sometimes technically difficult due to probe non-availability. Techniques such as CGH and interphase conversion are universal methods that could be used for most patients.

The work of the ESHRE PGD consortium, which has just commenced, will be important in the future of PGD, collating data and reporting on various aspects of PGD treatment worldwide.

CONCLUSION

PGD has already helped many couples worldwide to achieve a healthy family. PGD has been thwarted by problems such as mosaicism and ADO which have led to misdiagnosis, but it is hoped that with our current knowledge reliable and efficient techniques can be developed and that many more couples can be treated using this procedure.

REFERENCES

ESHRE Preimplantation Genetic Diagnosis (PGD) Consortium (1999) Preliminary assessment of data from January 1997 to September 1998 ESHRE PGD Consortium Steering Committee. *Hum Reprod* **14**: 3138–3148.

ESHRE PGD Consortium (2000) Data Collection II (May 2000). *Human Reproduction* **15**: 2673–2683.

Griffin DK, Handyside AH, Penketh RJA, Winston RML & Delhanty JDA (1991) Fluorescent in situ hybridisation to interphase nuclei of human pre-implantation embryos with X and Y chromosome specific probes. *Hum Reprod* **6**: 101–105.

Griffin DK, Handyside AH, Harper JC *et al.* (1994) Clinical experience with preimplantation diagnosis of sex by dual fluorescent in situ hybridisation. *J Assist Reprod Genet* **11**: 132–143.

Handyside AH, Kontogianni EH, Hardy K & Winston RM (1990) Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* **344**(6268): 768–770.

Hardy K, Martin KL, Leese HJ, Winston RML & Handyside AH (1990) Human preimplantation development in vitro is not adversely affected by biopsy at the 8-cell stage. *Hum Reprod* **5**(6): 708–714.

Verlinsky Y, Cieslak J, Ivakhnenko V *et al.* (1996) Birth of healthy children after preimplantation diagnosis of common aneuploidies by polar body fluorescent in situ hybridization analysis. *Fertil Steril* **66**: 126–129.

Verlinsky Y & Kuliev A (1998) Preimplantation genetics. *J Assist Reprod Genet* **15**: 215–218.

2 Genetic Basis of Inherited Disease

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INTRODUCTION

Before considering the approaches to preimplantation genetic diagnosis (PGD) it is important to have a clear understanding of the genetic basis of inherited disease. Inherited disease may be caused by mutation or loss of a single gene or by chromosomal rearrangement carried by a parent which leads to the production of genetically unbalanced gametes. A newly emerging category of disorders that presents particular problems for PGD is those that are caused by a variable increase in the number of copies of a particular trinucleotide repeat sequence (i.e. three base pairs, for example cytosine, guanine, guanine—CGG—and their paired bases on the opposite DNA strand) within the vicinity of a gene.

GENE MUTATION

Mutation simply means change. Change in gene function can be brought about by loss of the whole or part of the coding sequence of the gene (deletion) or by alteration in the bases that make up the DNA molecule (substitution). More rarely, gene duplication can cause disease. Surrounding each gene are modifiying sequences that affect gene expression; these too can mutate. Mutations also affect non-coding DNA sequences, usually regions of repetitive DNA that are associated with each gene. Mutations in these regions are unlikely to cause disease. Several forms of these variants may exist in the population and if they are common enough to be found in at least 1% of people they are known as polymorphisms. Polymorphisms are very useful as genetic markers since a particular variant will tend to be inherited along with the gene mutation in each family.

MENDELIAN INHERITANCE OF SINGLE GENE DISORDERS

The relevant genes may be carried either on the X chromosome or on the non-sex chromosomes (the autosomes). Autosomal conditions may be described as 'dominant' or 'recessive'. At the outset it is important to remember that chromosomes exist in pairs, one from each parent, which in turn means that a gene for a particular character is also present twice in each cell. The exception is the X

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chromosome in males, since the Y chromosome is much smaller and carries few expressed genes. The Y chromosome carries genes important for sex determination and fertility; loss of function of these genes is therefore harmful.

Examples of pedigrees with these typical patterns of inheritance are shown in Figure 2.1. An autosomal recessive mode of inheritance is suggested when there are affected children in a family with no known history of the disease. This happens when a gene carrier 'marries' into the family. In dominant conditions affected children are seen in every generation since the parent has a one in two chance of passing on the condition irrespective of the genetic status of his/her partner. In families with an X-linked recessive condition only males are affected and they may appear in every generation because some of the mothers will be carriers of the gene, but there is no male-to-male transmission.

AUTOSOMAL RECESSIVE

The majority of severe single gene disorders are recessive. This means that a person who carries one abnormal copy of a particular gene in each of their cells along with one normal copy on the other chromosome will not manifest the disease. This person is a heterozygous carrier. If their partner is not a carrier they can safely produce children who will be unaffected. If by chance their partner is also a carrier for the same abnormal gene then they have a one in four chance of having a child who has both copies of the abnormal gene. This child will then be affected by the disease in question. On average, half their children will be gene carriers like themselves and a quarter will have two normal genes (Figure 2.2a).

The commonest autosomal recessive disorder in Caucasian populations is cystic fibrosis (CF); this is the cause of frequent requests for PGD. The most common fault (mutation) in this gene is a 3 base pair deletion known as ΔF508; this means that in most cases both parents of an affected child are likely to be carrying this mutation. This situation makes single cell diagnosis relatively straightforward. However, there are a number of rare CF mutations that are not so easily detected. If the parents carry different mutations the child who inherits these two different abnormal genes will be affected; such a child is called a 'compound heterozygote' (Figure 2.2b).

Worldwide, the commonest autosomal recessive disease is β-thalassaemia. In contrast to CF, there are numerous different common mutations in this gene. Certain types predominate in different populations, but it is still the case that most parents of affected children will be carriers of different mutations. This makes PGD more difficult because the affected embryos will be compound heterozygotes. Single cell diagnosis is also technically more demanding than for CF since most changes are substitutions of one base for another rather than missing or additional bases.

AUTOSOMAL DOMINANT

Autosomal dominant disorders require only one copy of the abnormal gene to be present in each cell for the disease to be expressed. The presence of a normal copy on the other chromosome is not sufficient to ensure normality. Generally, dominant

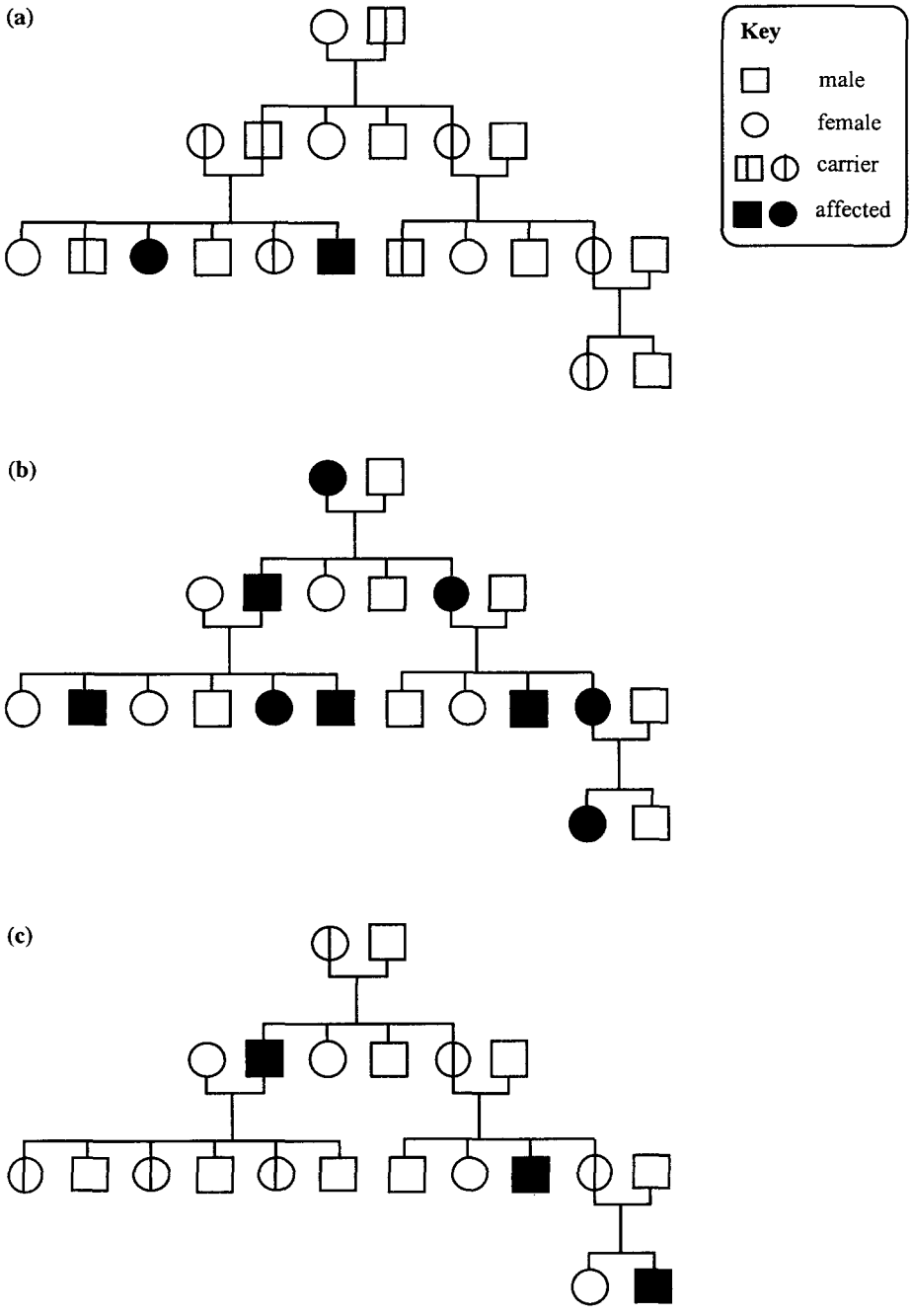

Figure 2.1 Modes of inheritance. (a) Autosomal recessive. (b) Autosomal dominant. (c) X-Linked recessive

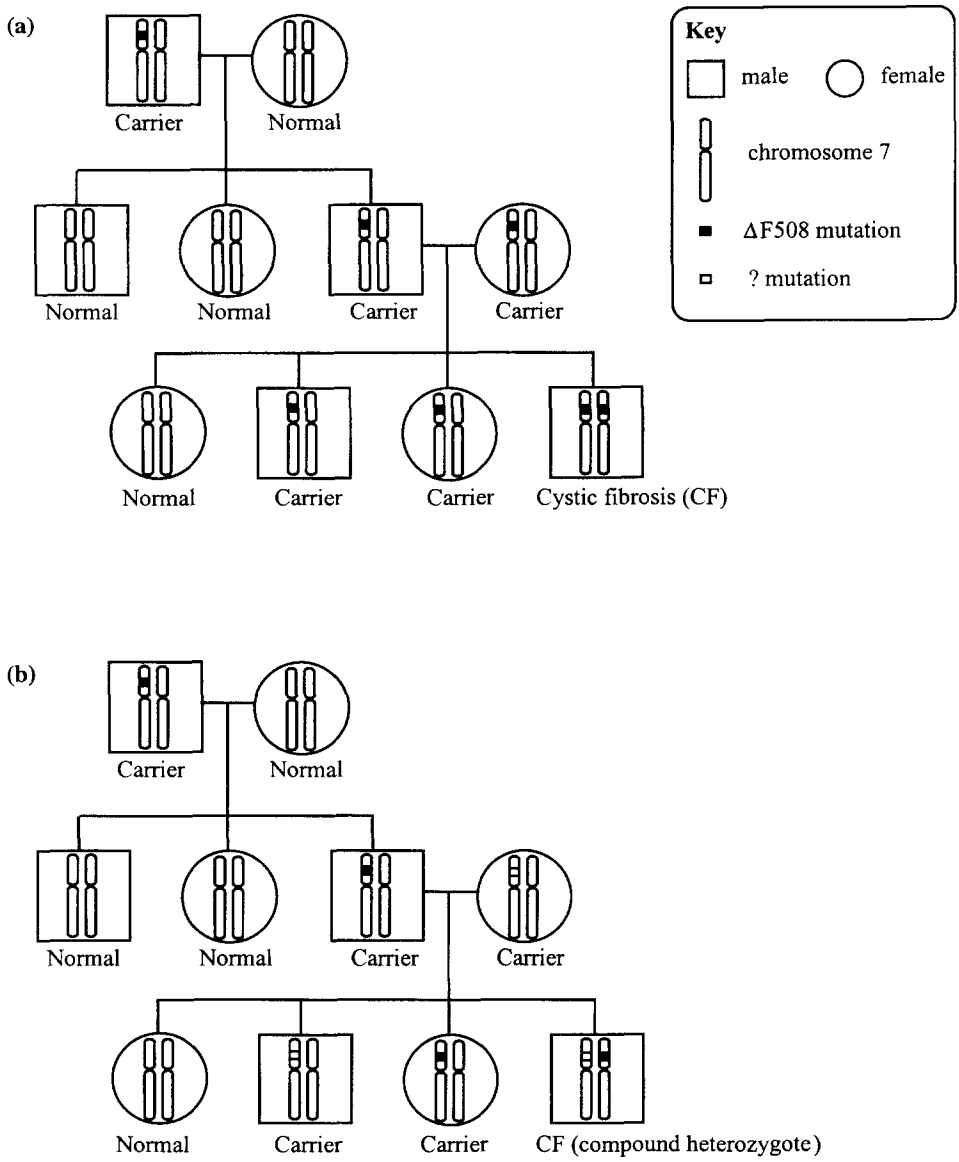

Figure 2.2 Cystic fibrosis family pedigree illustrating the typical inheritance of an autosomal recessive gene defect. (a) In this family the common cystic fibrosis (CF) mutation ΔF508 is passed through healthy carriers in the grandparents' and parents' generations to the grandchildren, one of which is affected by the disease. (b) In this case the father carries the ΔF508 mutation whilst the mother carries a different, much rarer mutation of the CF gene. The child affected by cystic fibrosis who has inherited both defective copies is essentially a carrier of each mutation and is termed a compound heterozygote

disorders are not so severe or life-threatening as recessive disorders. Usually, the gene carriers are able to have children either despite being affected, as in the case of achondroplasia (dwarfism), or because the disease itself is of late onset (e.g. Huntington's disease). Autosomal dominant disorders that have led to requests for PGD include Marfan's syndrome, which is a connective tissue disorder that predisposes to heart disease, and polyposis coli, in which the development of hundreds of adenomas in the colon makes eventual progression to colorectal cancer inevitable. On average, half the children of an affected mutant gene carrier will also be affected. PGD is technically demanding because it is vital to be able to detect both the mutant and normal copies of the gene in a single cell with equal efficiency. Many couples are willing to consider PGD for late onset disorders when they would not accept prenatal diagnosis and termination of an established pregnancy if the fetus were affected.

Dominant conditions may not always be fully 'penetrant', i.e. individuals who are gene carriers may not manifest the disease. In some cases we understand the reason; for instance in inherited cancer predispostion a second 'hit' knocking out the normal gene on the other chromosome in an appropriate cell is necessary for cancer to develop. The gene carrier may escape cancer if the second hit does not occur.

X-LINKED INHERITANCE

X-linked disorders (caused by mutation in genes that are carried on the X-chromosome) can be either recessively or dominantly inherited. In practice, almost all severe examples are recessives and are carried by females who are themselves unaffected or only mildly so, because of the normal copy of the gene on their second X chromosome. Half their sons (who get their single X chromosome from their mother) will be normal; however, the others will be affected with the disease as their Y chromosome will not have the normal gene. Common X-linked recessive disorders include Duchenne muscular dystrophy and haemophilia. Since the molecular basis of these two diseases is understood it would be theoretically possible to carry out a specific PGD and diagnose affected males. However, the exact gene change is not always known in particular families so that in practice it may be easier to offer sexing of the embryo with the transfer of females. Added to this is the fact that there are well over 200 known X-linked disorders and for most of them the molecular basis is not known. For these families, all that can be offered in the way of prenatal diagnosis is the sexing of the fetus and termination of all male pregnancies, half of which will be unaffected. PGD offers the chance to avoid this scenario.

POLYGENIC INHERITANCE

Disorders such as diabetes and schizophrenia are caused by the interaction of many different genes together with environmental influences. Technically it is not possible to offer PGD for this type of condition at present since the genetic basis is not fully understood and the exact environmental component is unknown.