

Prenatal diagnosis of common single gene disorders by DNA technology

V Chan, TK Chan

Using the new DNA technology, it is now possible to offer prenatal diagnosis or presymptomatic testing for many genetic diseases. For prenatal diagnosis, foetal tissue is obtained by chorionic villus sampling at 9 to 11 weeks gestation or amniocentesis at 18 weeks. The programme in Hong Kong, which started in 1982, is reviewed here and now includes α and β thalassaemia, haemophilia A and B, Duchenne muscular dystrophy, Huntington's disease, and spinal muscular atrophy. DNA diagnosis can now be performed using a single cell obtained from pre-implantation embryos or from rare foetal cells isolated from maternal peripheral blood. The latter is safer and more acceptable to parents. Presymptomatic testing for untreatable diseases such as Huntington's disease poses new ethical and social problems that need to be resolved. As many more genes are being discovered, prenatal diagnosis and presymptomatic testing programmes will continue to meet new challenges in the future.

HKMJ 1997;3: 173-8

Key words: Prenatal diagnosis; Carrier testing; Genetic screening; Heterozygote detection

Introduction

The advent of DNA technology in the past 15 years has advanced our understanding of the molecular pathology of many diseases. This knowledge has been applied not only to devising new treatment modalities and designing new therapeutics with recombinant DNA techniques, but also to the improvement of diagnostic tests. Many diseases were previously diagnosed by biochemical means, which sometimes proved to be non-specific due to the presence of various isoforms or sources of the enzyme, as in Duchenne muscular dystrophy¹ or yielded equivocal results due to X-inactivation, in the case of carrier testing for haemophilia.² The use of DNA techniques to define the molecular defects of disease has enabled specific, accurate, and more sensitive diagnostic tests to be developed.

Genetic diseases account for 20% of the deaths during the newborn period and a higher percentage of

serious morbidity in infancy and childhood.³ The incidence and severity of single gene diseases varies in different ethnic groups and may occur in up to 2% of newborns.⁴ Besides the high financial cost incurred in the management of such newborns, and for some throughout life, the physical and psychological sufferings of those affected and their families are immeasurable. Before there is any radical cure for such diseases by gene therapy, prenatal diagnosis and prevention through therapeutic abortion of affected foetuses, is a useful option. For diseases that manifest later in life, such as Huntington's disease, predictive testing in an adult may facilitate their planning for the future and prenatal diagnosis should be advocated to prevent the birth of further affected progeny.^{5,6}

Prenatal diagnosis programme in Hong Kong

Prenatal diagnosis for α and β thalassaemia was first established in Hong Kong in 1982. This was a collaborative effort of the DNA Diagnostic Laboratory of the University Department of Medicine, Queen Mary Hospital, and the Wu Chung Prenatal Diagnosis Laboratory of the University Department of Obstetrics and Gynaecology, Tsan Yuk Hospital. Over the past 14 years, the DNA Diagnostic Laboratory has had

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong
V Chan, PhD, FRCPath
TK Chan, MD, FRCP

Correspondence to: Prof V Chan

to cope with the increasing repertoire of genetic defects requiring diagnosis as well as the evolving new DNA technologies. The introduction of the polymerase-chain reaction (PCR) in 1985^{7,8} revolutionised the whole approach to DNA diagnostics in the 1990s. The ability to amplify a gene of interest one million-fold *in vitro* (Fig 1) has simplified and expanded the scope of DNA diagnostics. To date, more than 1200 prenatal diagnoses have been made and 235 affected pregnancies confirmed (Fig 2). This review summarises the approaches used in the prenatal diagnosis of common inherited single gene diseases in Hong Kong.

The thalassaemias

The thalassaemias (thal) [α and β] are the most common inherited diseases in Southeast Asia and the Mediterranean region. Within Southeast Asia, the incidence for both types of thal trait ranges from 1% to 20% in various regions. Couples who are both α or β thal trait (carriers) run a 25% risk of having a homozygous foetus with each pregnancy. Such a foetus would either die *in utero* in late pregnancy or at birth, as in homozygous α thal 1 (Hb Barts hydrops foetalis), or develop severe anaemia in infancy and become transfusion-dependent throughout life, as in the case of homozygous β thal. Many places in the region, e.g. Hong Kong, Singapore, and Australia have established prenatal diagnosis programmes. Couples at risk are screened at the antenatal clinic and premarital clinic and counselled as to the mode of inheritance of the disease, management of the severely affected child, and the reproductive options available to them.

Prenatal testing of α thal is done mainly by the detection of α genes in foetal DNA extracted directly from chorionic villus or amniocytes.⁹ For most service laboratories, Southern blotting or dot blot is still the preferred method because of the risk of a false negative associated with the PCR amplification of the α thal 1 chromosome. Contamination of 0.01% maternal or normal DNA template will give a false result.

For β thal, there are at least 12 known β thal mutations in the southern Chinese population.¹⁰ An earlier method of direct mutation detection involves the hybridization of allele-specific oligonucleotide (ASO) probes to PCR-amplified β gene in the test sample, which was dotted onto nylon strips. This requires hybridization to 24 different probes (both normal and mutant probe for each of the 12 defects) in the screening procedure. A recent improvement is the reverse dot blot (RDB) technique,¹¹ whereby the sets of 12 ASO probes pertaining to the normal and mutant sequences,

respectively, are each dotted onto a single nylon strip, and hybridization is made with the amplified β gene in solution. Both 'normal' and 'mutant' strips are hybridized and washed simultaneously. This allows simultaneous screening for the molecular defect within the family and prenatal diagnosis for the foetus in a single step. It is now possible to obtain a result for β thal prenatal diagnosis within five to six hours of foetal sampling. For those with unknown molecular defects, foetal blood sampling and analysis of the β/γ globin chain ratio¹² is the obvious option.

The haemophilias

Haemophilia A, the most common congenital bleeding disorder, affects 1:10 000 live-born males. Females are usually only carriers of the disease, thus carrier testing and prenatal diagnosis, if required, should be offered to those with a positive family history. Forty per cent of severe haemophilia A arises as a result of intrachromosomal meiotic recombination between an F8 associated gene (F8A gene) in intron 22 of the FVIII gene itself and either one of two copies of the same gene at the telomere of the X chromosome.¹³ This defect can be detected by Southern blotting of Bcl I digested genomic DNA and hybridization with an intron 22 probe.¹³ Thus the detection of intron 22 inversion is the foremost strategy in diagnosis.¹⁴ Given the huge size of the FVIII gene (186 kb), detection of other types of molecular defect is somewhat labour intensive.¹⁵ Linkage of the affected gene by restriction fragment length polymorphism (RFLP) at the common intragenic sites, e.g. Bcl I, Xba I/Kpn I, and extragenic sites (Taq I - St 14), or microsatellite repeat polymorphism (MRP) at the intron 13 and intron 22 loci is the alternate approach.¹⁶ All of the southern Chinese haemophilia A families in this region would be able to be diagnosed by linkage analysis.¹⁷ Recent improvements in diagnosis include the development of a semi-automated procedure using fluorescein dye-labelled primers for PCR and analysis of the PCR product on an automated DNA sequencer.¹⁸ Laser quantitation of fluorescence and dedicated computer software (Genescan, Applied Biosystems Inc., Foster City, Ca, US) provide added sensitivity and rapid haplotype analysis.

The incidence of haemophilia B is approximately half that of haemophilia A. It usually manifests a milder phenotype and therefore few cases may present at clinics or hospitals, giving the impression of a much lower incidence. The FIX gene is much smaller in size (33.5 kb) with a cDNA of 1.4 kb only. This small size makes direct detection of the mutation feasible by PCR of

the exons and direct genomic sequencing.¹⁹ Since Chinese and other Orientals lack heterozygosity for the common RFLP sites, detection of the mutation is the standard strategy in diagnosis and carrier testing for these ethnic groups.¹⁹ In Caucasians, various intragenic RFLPs are available for linkage studies^{16, 20} in addition to direct mutation detection.

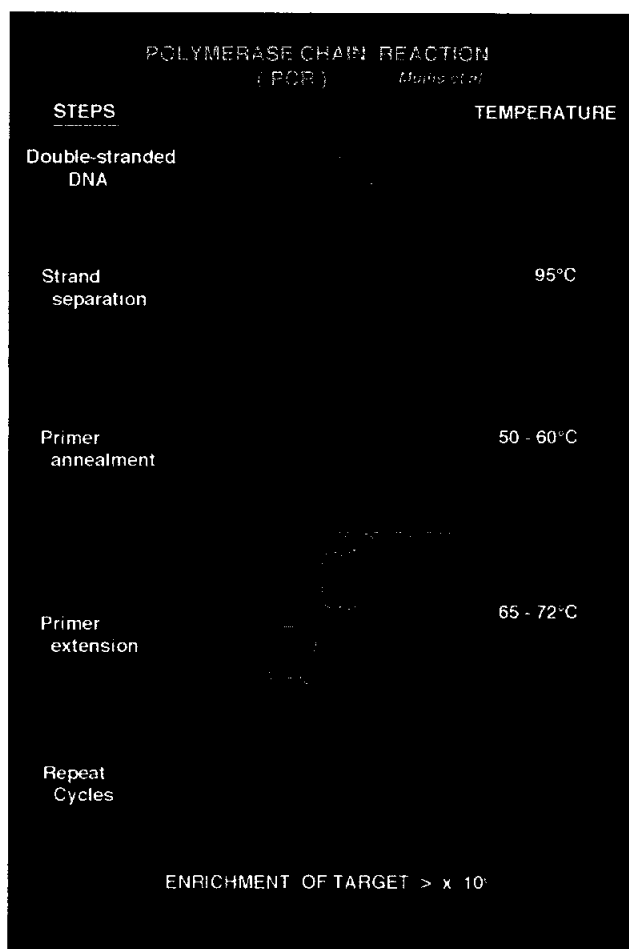


Fig 1. Schematic diagram showing the steps involved in the polymerase chain reaction (PCR)

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) and its milder form, Becker muscular dystrophy (BMD), are caused by mutation of the dystrophin gene located on the short arm of the X chromosome at Xp21. This X-linked recessive disorder affects 1:3500 live-born males.²¹ Affected DMD individuals usually present with proximal muscular weakness in early childhood, with loss of ambulation by 12 to 15 years of age and eventual death from respiratory failure in the third decade of life. Those with BMD have a milder phenotype and many are still mobile in mid-life (45-50 years). Recent gene therapy efforts using myoblast transplant

have not measured up to expectations.²² Hence, prevention is still the mainstay of management for families at risk. With the isolation of the DMD gene by 'reverse genetics' in 1987,²³ prenatal diagnosis and carrier testing for this condition has become widely available. Unlike that, there is no established screening procedure for females at risk, except for the presence of increased serum creatinine kinase in approximately 60% of carriers.¹ Anyone with a family history of the disease will be eligible. Since the indexed patient may die within the third decade, storage of the Epstein-Barr virus transformed lymphocytes to establish a permanent cell line of the propositus is advisable.

Approximately 50% to 60% of DMD cases arise as a result of gene deletion. Direct detection of this type of defect can be made by PCR of deletion-prone exons or Southern blot hybridization with cDNA.^{24, 25} For other families, RFLP linkage can be made using a number (8-10) of intragenic sites.²⁶ In addition, a number of MRPs have been localised around the 3' end of the gene.^{27, 28} Multiplex PCR with fluorescein-labelled primers can be employed to provide a rapid linkage study. Since these MRPs are strategically located in the deletion-prone region of the DMD gene, failure to PCR is a positive indication of deletion defect. Furthermore, hemizyosity of the MRP in an at-risk female in these families would be indicative of carrier status.²⁹ As the DMD gene is 2000 kb in length, there is a risk of error due to meiotic recombination when RFLP is used to link the affected gene. To reduce this chance of error, multiple intragenic sites as well as sites on 5' and 3' flanking regions of the gene should be used.

Huntington's disease

Huntington's disease (HD) is a neurodegenerative autosomal dominant disorder that manifests in mid-life and presents with involuntary choreic movement, cognitive and psychiatric disturbances, and dementia. Currently, there is no effective treatment, cure, or means of arresting or delaying the inexorable progression to death, which usually occurs some 10 to 15 years after the onset of disease. The identification of the HD gene (IT5) and detection of the defect as an expansion of the 5' trinucleotide repeat sequence (CAG)_n,³⁰ have allowed more accurate diagnosis of the disease. With a disease that manifests in mid-life, the penetrance rate is usually high, since patients might have married and produced a family before the onset of illness. Presymptomatic testing of at-risk individuals and prenatal diagnosis are seen as welcome preventive measures to help stop passage of the disease to future gen-

erations.³¹⁻³³ However, various ethical and psychosocial issues need to be considered. Since the condition is untreatable, testing and results should be requested by and only made available to concerned individuals after adequate counselling has been given.

Spinal muscular atrophies

Spinal muscular atrophies (SMAs) are a group of common, fatal, autosomal recessive disorders, with an incidence of 1:6000 newborns. They are characterised by degeneration of the anterior horn cells of the spinal cord, leading to weakness and wasting of the voluntary muscles, and paralysis. Childhood SMAs are classified into three types, depending on the age of onset and clinical course. Type I (Werdnig-Hoffman) is the most severe, with clinical onset before six months of age, death occurring in the first year, and those affected never being able to sit unaided. Type II is of intermediate severity; patients can usually sit unaided and survive beyond 10 years. Type III (Kugelberg-Welander) represents a mild form with symptoms manifesting in infancy or youth and the individual being able to walk unaided. The SMA candidate gene region was mapped to the long arm of chromosome 5 (5q 11.2 - q 13.3) by linkage analysis and more recently, three candidate genes have been re-

ported.³⁴⁻³⁶ Two copies of the survival motor neuron gene (SMN) were found, one centromeric (cBCD 541) and the other telomeric (SMN), differing from each other by one nucleotide in exon 7 and exon 8, respectively. Homozygous deletion of both exons 7 and 8 or exon 7 only in the SMN gene was found in 96% of type I SMA, 94% of type II, and 82% of type III, as well as in 0.3% of SMA parents³⁷ and a PCR-based method has been described for its detection.³⁸

In addition, homozygous deletion of another nearby gene, the neuronal apoptosis inhibitory protein gene (NAIP), was also observed in approximately 50% of type I and 18% of types II and III patients.³⁷ Point mutation of the SMN gene may account for the molecular pathology in 10% of cases without deletion; three types of point mutation have been reported. This high frequency of homozygous SMN gene deletion in SMA patients and its virtual absence in control subjects makes it a likely cause of SMA. The finding of homozygous deletion in unaffected siblings of about 10% of SMA families, however, makes presymptomatic testing difficult, since it implies that deletion is not necessarily associated with disease and other determining factors may play a role.³⁷ These points should be considered in genetic counselling.

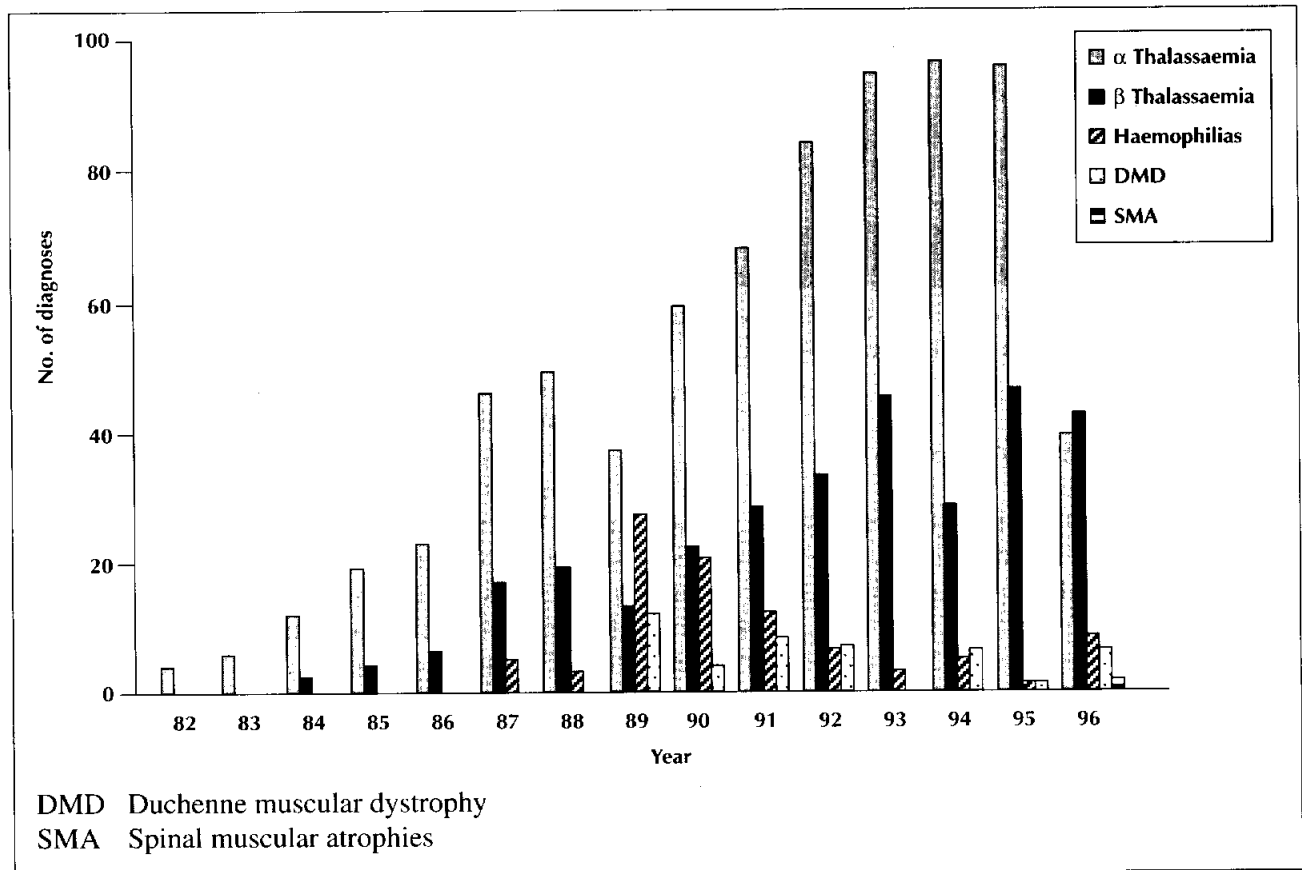


Fig 2. DNA-based prenatal diagnosis currently performed in Hong Kong

Future prospects

With the genetic mapping and cloning of disease genes, our understanding of the genetic basis of diseases will increase and with it, the means to offer accurate presymptomatic and carrier testings as well as prenatal diagnosis. Commensurate with this, is the improvement in the techniques of procuring foetal tissue. First trimester diagnosis can be made using CVS obtained at 9 to 11 weeks, and although a two to three per cent incidence of abortion^{39,40} is recorded at this gestation period, this cannot be ascribed entirely to the procedure, as miscarriage of problematic pregnancies tends to occur in early gestation.⁴⁰ Amniocentesis is only associated with a 0.5% risk of abortion,⁴¹ but is usually performed at 18 weeks gestation. Thus termination of an affected pregnancy, diagnosed after amniocentesis, cannot be made until the second trimester. This obviously is in itself associated with a higher risk compared to first trimester termination. Currently, much effort is placed on the isolation of foetal cells from maternal peripheral blood.^{42,43} If successful, this will provide a non-invasive prenatal diagnosis with no inherent risk to the foetus. Meanwhile, highly sensitive and specific tests are being developed for use in single cell analysis^{44,45} and these can be applied to pre-implantation diagnosis as well as to rare foetal cells harvested by non-invasive procedures from maternal blood.

References

- Lane RJ, Roses AD. Variation of serum creatinine kinase levels with age in normal females: implication for genetic counselling in Duchenne muscular dystrophy. *Clin Chim Acta* 1981;113:75-86.
- Green PP, Mannucci PM, Briet E. et al. Carrier detection in haemophilia A: a cooperative international study II. The efficacy of a universal discrimination. *Blood* 1986;67:1560-7.
- Centers for Disease Control. Contribution of birth defects to infant mortality. *MMWR* 1989;38:633-5.
- Polani PE. Prenatal diagnosis and genetic screening. In: Royal College of Physicians Report. London, 1989.
- Craufurd D, Tyler A. Predictive testing for Huntington's disease: protocol of the UK Huntington's Prediction Consortium. *J Med Genet* 1992;29:915-8.
- European Community Huntington's Disease Collaborative Study Group. Ethical and social issues in presymptomatic testing for Huntington's disease: a European Community collaborative study. *J Med Genet* 1993;30:1028-35.
- Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of b globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 1985;230:1350-4.
- Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;239:487-91.
- Chan V, Ghosh A, Chan TK, Wong V, Todd D. Prenatal diagnosis of homozygous alpha thalassaemia by direct DNA analysis of uncultured amniotic cells. *BMJ* 1984;288:1327-9.
- Chan TK, Chan V. Molecular genetics and prenatal diagnosis of common inherited diseases. *CME Review*, National University Hospital, Singapore 1991;1:67-77.
- Chehab FF. Molecular diagnostics: past, present and future. *Hum Mutat* 1993;2:331-7.
- Kan YW, Golbus M, Trecartin R, Furbetta M, Cao A. Prenatal diagnosis of homozygous β -thalassaemia. *Lancet* 1975;2:790-2.
- Lakich D, Kazazian AH Jr, Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nat Genet* 1993;5:236-41.
- Goodeve AC, Preston FE, Peake IR. Factor VIII gene rearrangements in patients with severe haemophilia A. *Lancet* 1994;343:329-30.
- Chan V, Pang A, Chan TPT, Chan VWY, Chan TK. Molecular characterization of haemophilia A in Southern Chinese. *Br J Haematol* 1996;93:451-6.
- Peake IR, Lillicrap DP, Boulyenkov V, et al. Report of a joint WHO/WFH meeting on the control of haemophilia: carrier detection and prenatal diagnosis. *Blood Coagul Fibrinolysis* 1993;4:313-44.
- Yip B, Chan V, Chan TK. Intragenic dinucleotide repeats in factor VIII gene for the diagnosis of haemophilia A. *Am J Hematol* 1994;88:889-91.
- Yip B, Chan V, Chan TK. A semi-automated method for analysis of intron 13 and intron 22 dinucleotide repeat polymorphisms of the factor VIII gene. *Clin Lab Haematol* 1996;18:111-4.
- Chan V, Yip B, Tong TMF, et al. Molecular defects in haemophilia B: detection by direct restriction enzyme analysis. *Br J Haematol* 1991;79:63-9.
- Winship PR, Brownlee GG. Diagnosis of haemophilia B carriers using intragenic oligonucleotide probes. *Lancet* 1986;2:218-9.
- Dubowitz V. Muscle disorders in childhood. In: Major problems in clinical paediatrics. Vol. 16. Philadelphia: Saunders, 1978:19-69.
- Morandi L, Bernasconi P, Gebbia M, et al. Lack of mRNA and dystrophin expression in DMD patients three months after myoblast transfer. *Neuromuscular Disord* 1995;5:291-5.
- Koenig M, Hoffman EP, Bertelson CJ. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell* 1987;50:509-17.
- Henteman M, Reiss J, Wagner M, Cooper DN. Rapid detection of deletions in Duchenne muscular dystrophy gene by PCR amplification of deletion-prone exon sequences. *Hum Genet* 1990;84:228-32.
- Roberts RG, Bentley DR, Barby TF, Manners E, Borrow M. Direct diagnosis of carriers of Duchenne and Becker muscular dystrophy by amplification of lymphocyte RNA. *Lancet* 1990;2:1523-6.
- Chan V, Chan TK. Prenatal diagnosis and carrier testing of Duchenne muscular dystrophy by DNA analysis. *J HK Med Assoc* 1991;43:83-7.
- Beggs AH, Kunkel LM. A polymorphic CACA repeat in the 3' untranslated region of dystrophin. *Nucleic Acids Res* 1990;18:1931.
- Clemens PR, Fenwick RG, Chamberlain JS, et al. Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families, using dinucleotide repeat polymorphism. *Am J Hum Genet* 1991;49:951-60.
- Yip B. The uses of short tandem repeats in the diagnosis of genetic diseases. Hong Kong: University of Hong Kong; 1996. Thesis.

30. Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosome. *Cell* 1993;72:971-83.
31. Duyao M, Ambrose C, Myers R. Trinucleotide length instability and age of onset in Huntington's disease. *Nat Genet* 1993;4:387-92.
32. Snell RG, MacMillan JC, Chendle JP. Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. *Nat Genet* 1993;4:393-7.
33. Chan V, Yu YL, Chan TPT, et al. DNA analysis of Huntington's disease in southern Chinese. *J Med Genet* 1995;32:120-4.
34. Lefebvre S, Burglen L, Reboullet S, et al. Identification and characterization of a spinal muscular atrophy determining gene. *Cell* 1995;80:155-65.
35. Roy N, Mahadevan MS, McLean M, et al. The gene for neuronal apoptosis inhibitor protein (NAIP), a novel protein with homolog to baculoviral inhibitors of apoptosis, is partially deleted in individuals with type 1, 2 and 3 spinal muscular atrophy (SMA). *Cell* 1995;80:167-78.
36. Thompson TG, DiDonato CJ, Simard LR, et al. A novel cDNA detects homozygous microdeletions in greater than 50% of type 1 spinal muscular atrophy patients. *Nat Genet* 1995;9:56-61.
37. Hahnen E, Forkett R, Marke C, et al. Molecular analysis of candidate genes on chromosome 5q13 in autosomal recessive spinal muscular atrophy: evidence of homozygous deletions of the SMN gene in unaffected individuals. *Hum Mol Genet* 1995;4:1927-33.
38. van der Steege G, Grootsholten PM, van der Vlies P, et al. PCR-based DNA test to confirm clinical diagnosis of autosomal recessive spinal muscular atrophy. *Lancet* 1995;345:985-6.
39. Ward RH. Techniques of chorion villus sampling. *Bailliere's Clinical Obstet Gynaecol* 1987;1:489-511.
40. Rhoads GG, Jackson LG, Schlesselman SE, et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. *N Engl J Med* 1989;320:609-17.
41. Leschot NJ, Verjaal M, Treffers PE. Risk of midtrimester amniocentesis: assessment in 3000 pregnancies. *Br J Obstet Gynaecol* 1985;92:804-7.
42. Simpson JL, Elias S. Isolating fetal cells from maternal blood. *JAMA* 1993;270:2357-61.
43. Tutschek B, Sherlock J, Halder A, Delhanty J, Rodek CF, Adinolfi M. Isolation of fetal cells from transcervical samples by micromanipulation: molecular confirmation of their fetal origin and diagnosis of fetal aneuploidy. *Prenatal Diagn* 1995;15:951-60.
44. Liu J, Lissens W, Silber SJ, Devroey P, Liebaers I, Van-Steirteghem A. Birth after pre-implantation diagnosis of the cystic fibrosis delta F508 mutation by polymerase chain reaction in human embryos resulting from intracytoplasmic sperm injection with epididymal sperm. *JAMA* 1994;272:1858-60.
45. Chan V, Yip B, Lau K, Chan TK. A semi-automated allele specific PCR for detection of β thalassemia mutations [abstract]. *Int J Hematol* 1996;64(1 Suppl):298.