Review

Potential of molecular diagnosis for early detection of disability

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Introduction

Disability resulting from both acquired and genetic causes affects a fairly large proportion of the population in industrialized countries. A study of the demographics of disability in the United States of America estimated that 43 million people have a disability, defined by the Disabilities Act of 1990 as "Limitation in actions or activities because of a physical, mental or other health condition" [*1*]. Such surveys have yet to be conducted in developing countries to assess the scale of the problem. However, from various reports of the World Health Organization (WHO) and other studies, it is apparent that disability prevalence is quite high in such countries.

Causes of disability

Disability may result from a variety of acquired or genetic causes (Box 1). It may be obvious at birth or may appear later in life [*2*]. The etiological factors in the development of disability may be present at preconception, prenatal, natal and postnatal levels.

As disability is chronic in nature, its early detection and early care and management may arrest the disabling process or ameliorate the complications resulting from the disabling process. The detection or diagnosis takes into consideration the etiological factors, and separate protocols are followed depending on whether the disability is acquired or genetic [*3,4*].

Acquired disability

Acquired disability due to trauma, infections, surgery, endocrine abnormalities or nutritional deficiencies can be recognized by taking a history of the patient and helped by physical and clinical examination. Laboratory confirmation can help the physician arrive at a final diagnosis. In general, the majority of the acquired disabilities can be easily diagnosed at an early stage and appropriate measures of intervention and management can be adopted accordingly.

Genetic disability

Genetic disorders are a frequent etiology of disability, where a wide spectrum of genetic disorders are the known causative factors of a variety of disabilities. Some of the disorders are obvious and present at birth, while others appear later, either soon after birth or after a few years. These disorders are either single gene disorders (autosomal

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Box 1 **Causes of disability**

Acquired	Genetic
Infections	Single
Trauma	Autosomal recessive
Surgery	Autosomal dominant
Exposure to drugs or chemicals	Sex linked
Exposure to toxins	Chromosonal
Nutritional deficiencies	Numerical
Acquired endocrine abnormalities	Structural
Other	Multifactorial
	Mitochondrial

recessive, autosomal dominant, X-linked recessive or X-linked dominant), chromosomal (numerical or structural), multifactorial or mitochondrial.

The normal human genome. In the nuclei of human cells, the genetic information is stored on 23 pairs of chromosomes; one chromosome from each pair is inherited from the maternal and the other from the paternal side. Each chromosome is made up of deoxyribonucleic acid (DNA) packed with proteins known as histones. The genetic information is present on the DNA in the form of specific sequences of bases. Between 50 000 and 100 000 genes are located on the set of 23 chromosomes inherited from one parent, and on exactly the same positions (loci), genes determining the same character are present on the set of 23 chromosomes inherited from the other parent. Hence for each characteristic (i.e. height, hair colour, skin colour, every protein in the body) each person has two alleles. If the genes are exactly the same, their condition is referred to as homozygous, while if one is different from the other in one or more bases the condition is said to be heterozygous.

Mutations. The phenomenon that brings about the changes in the base sequences in the genes is known as mutation. These changes are generally permanent and are brought about either spontaneously or on exposure to certain mutagenic agents, such as X-rays, ultraviolet rays, azo dyes, certain drugs, certain viruses and chemicals. The mutation may change a single base pair, cause partial or complete gene deletion, gene expansion, gene rearrangement or even chromosome rearrangement. Some mutations are very harmful and produce a genetic disorder often associated with disability, others have very little effect and still others are beneficial as they result in the production of forms which are more resistant to the environmental conditions.

The disease diagnosis is drawn from physical and clinical examination, and confirmed by laboratory tests. Recently, with the advent of recombinant DNA technology, the detection of carriers (i.e. heterozygotes), who are generally clinically asymptomatic, has been made possible [*5–10*].

DNA technology and detection of disability

Recently, recombinant DNA technology has enabled the diagnosis of a large number of diseases, examples of which are given in Box 2. The main advantage of DNA technology is the small samples required and the ways and means by which early detection can be achieved.

The methods may be applied at preconception, prenatal, neonatal or postnatal stages [5]. The methods applied for diagnosis depend on whether the gene and the nature of the mutation is known or if the gene is not known. In the former, a direct method is used, while in the latter an indirect method must be used.

Direct detection of genetic defects

In the situation where the gene is known, a direct detection of the abnormality can be made through the recognition of the mutation, as a single point mutation, a partial or complete deletion, a gene expansion, gene rearrangement or chromosome rearrangement [11].

Restriction endonucleases

The application of DNA technology to disease diagnosis has been possible since the discovery of a group of enzymes known as restriction endonucleases (RE) produced by prokaryotic cells. These enzymes cleave double-stranded DNA at a specific sequence of bases, thus producing DNA fragments. The fragments of DNA generated are then separated by electrophoresis on the basis of size differences. Thereafter, the fragments are transferred to nitrocellulose or Hybond N sheets by the Southern blot method, denatured (made single-stranded) and treated with a labelled probe. The probe hybridizes to the complementary DNA fragment only, and after washing the excess

Box 2 Examples of genetic disabilities diagnosed by DNA technology

- Adenosine deaminase deficiency
- Adrenal hyperplasia
- α-antitrypsin deficiency
- Alport disease
- Antithrombin III deficiency
- Colour blindness
- Cystic fibrosis
- Ehlers–Danlos syndrome
- Friedreich ataxia
- Gaucher disease
- Glucose-6-phosphate dehydrogenase deficiency
- Gyrate atrophy
- Haemophilia A and B
- Hunter syndrome
- Hypothyroidism
- Hypoxanthine guanine phosphoribosyl-transferase deficiency
- Lesch–Nyhan syndrome
- Maple syrup disease
- Marfan syndrome
- Muscular dystrophy
- Myotonic dystrophy
- Neurofibromatosis
- Ocular albinism
- Osteogenesis imperfecta
- Phenylketonuria
- Polycystic kidney disease
- Porphyria
- Sickle-cell disease
- Skeletal dysplasia X-linked
- Tay–Sachs disease
- Testicular feminization syndrome
- Thalassaemias
- Others

probe, the DNA fragment that is complemented by the probe, i.e. the "index", is detected using autoradiography [*7–9*]. This procedure is one of the most widely used and is known as Southern blotting [*12*]. This type of restriction endonuclease analysis has been used for the detection of point mutations, gene deletions, gene rearrangement and chromosome fragmentation.

a) Detection of point mutations

Several disabilities are due to genetic defects resulting from a single point mutation. The mutation may be detected using restriction endonucleases, DNA sequencing techniques, dot blot analysis, denaturing gradient gel electrophoresis (DGGE), single-stranded conformational polymorphism (SSCP) or by other procedures. The restriction endonuclease site may be lost or a new site may be produced as a result of a mutation. From the size of the fragments of DNA produced, the presence or absence of the mutation is identified [*8,9*].

This strategy is used for the diagnosis of several diseases, including sickle-cell mutation (β^S), where the *Mst*II recognition site (CCTGAGG) in β^A is altered when the 6th codon GAG is mutated to GTG. In a normal β^A individual a 1.15 kb fragment carrying the β-globin gene is obtained, but once the recognition site is lost a large 1.35 kb fragment is obtained in β^S homozygous cases (Hb SS) and both 1.15 kb and 1.35 kb fragments are obtained in heterozygotes.

b) Detection of gene deletion

If a gene is flanked by a recognition site for a restriction endonuclease, a DNA fragment of a specific size is obtained on Southern blotting. On the other hand, if part or all of the gene is deleted, the DNA fragment is either absent or is reduced in size. Duchenne muscular dystrophy, a frequent cause of physical disability, is caused by deletion or other mutations in the dystrophin gene located on the X chromosome. Recently, methods have been standardized for the detection of the gene deletion, although previously it was diagnosed by linkage analysis.

In patients with familial isolated growth hormone deficiency (IGHD), the growth hormone (GH) gene is flanked by *Bam*HI sites which are 3.8 kb apart [*13*]. On electrophoresis following *Bam*HI digestion, patients with IGHD do not have this fragment while heterozygotes have a 3.8 kb band of a low intensity. As IGHD is an autosomal recessive disorder, both parents must be carriers to have an affected child. This can be easily diagnosed by restriction endonuclease analysis. The same strategy is used for diagnosis of α-thalassaemia [*14*].

c) Detection of gene/DNA elongation/expansion

The expansion or elongation of the gene may result from a mutation converting the stop codon to a codon which can code for an amino acid. On the other hand, expansion in the DNA regions flanking the genes occurs by an increase in the number of repetitive DNA sequences. This has been shown to cause diseases such as fragile X mental retardation and several others which lead to disability (Table 1). This is the most common cause of inherited mental retardation with an incidence of 1 in 1000–1500 males and 1 in 2000–2500 females. Cytogenetic studies can be used to diagnose the condition; however, these techniques are not very reliable for the detection of the carrier state in males and females [*15*].

The fragile X mutation is the result of an increase in the size of a CGG repeat segment of DNA in the 5' region of the *FMR-1* gene [*16–20*]. In a normal person, the length of this region is ≤ 150 bp, while in carriers it is around 200–600 bp. This condition in the carriers is referred to as pre-

mutation and its appearance precedes full mutation in subsequent generations. About 20% carrier males and 35% carrier females have some degree of mental impairment. Expansion > 600 bp is referred to as full mutation and is associated with full expression of mental retardation. This can be easily detected by Southern blotting, where a larger fragment containing the CGG repeat is obtained compared with the normal. Carrier detection can be achieved with certainty. A list of other diseases that can be diagnosed by the same strategy is presented in Table 1.

d) Detection of gene/chromosome rearrangement

Several genetic defects result from chromosomal rearrangements, which are often associated with gene rearrangement. In some haemophilia cases an intragenic inversion disrupts the factor VIII gene, and thus the gene product is not factor VIII, which leads to haemophilia [*21*]. Similarly, in a large number of acquired cancers, the basic defect is a specific chromosomal rearrangement which can be detected by Southern blotting. In patients with chronic granulocytic leukaemia, a reciprocal translocation occurs from chromosome 9q34 to 22q11 and results in the formation of Philadelphia (ph I) chromosome. The breakpoint is within the *abl* proto-oncogene on chromosome 9q34 and a break cluster region gene on chromosome 22q11. These result in a fusion which produces an oncogene. On Southern blotting a larger fragment (5 kb) and two normal 4.4 kb and 2.3 kb fragments are obtained on digestion with *Bgl*II, while in a normal person only 4.4 kb and 2.3 kb fragments are obtained [*22*].

e) Detection of chromosomal abnormality

Chromosomal abnormalities, both structural and numerical, can also be diagnosed using Southern blotting [*23*]. Some cases of Turner syndrome have chromosome fragments produced as a result of X chromosome or Y chromosome deletion. The presence of Y chromosomal material predisposes to gonadoblastomas. The presence of the Y chromosome fragment can be easily identified by Southern blotting and a Y-specific probe following *Eco*RI digestion. Turner syndrome patients with a Y fragment produce a 3.3 kb fragment.

Table 1 **Diseases leading to disability associated with expansion of triple repeats**

Disease	Trinucleotide repeat
Fragile X syndrome	CGG
Fragile X mental retardation	CGG
Friedreich ataxia	GAA
Myotonic dystrophy	CTG
Huntington disease	CAG
Spinal and bulbar muscular atrophy	CAG
Spinocerebellar ataxia (type I)	CAG

DNA amplification by polymerase chain reaction to detect mutation

Polymerase chain reaction (PCR) has had a tremendous impact on the usefulness of DNA technology as it allows a gene or a DNA fragment of interest to be amplified several thousand times [*24*]. PCR is a primer-directed enzymatic amplification of a specific DNA sequence. The primers for both sides of the gene or DNA to be amplified are selected, the DNA is denatured at 94 °C and the primers are annealed by lowering the temperature. DNA synthesis and extension at temperatures around 70 °C is achieved using *Thermus aquaticus* (Taq) polymerase. These steps, i.e. a complete

cycle, are repeated several times giving rise to the amplification of the desired fragment, where in each round the number of DNA fragments is doubled and after 30 cycles 10^6–10^7 copies are generated [*24*]. These can be directly visualized after staining with ethidium bromide following electrophoresis on agarose gel. The PCR product can be used for DNA sequencing, DGGE, SSCP, dot blot analysis, reverse dot blot analysis, amplification refractory mutation system (ARMS) and other techniques. With these techniques, the presence of a mutation can be localized and identified.

a) Detection of point mutations
The gene of interest is amplified and treated with the restriction endonuclease and the DNA fragments generated are separated by electrophoresis. The presence of a mutation alters the recognition site or produces a new recognition site and hence the fragments generated are of a different size in a mutated gene compared with a normal one. This is exemplified by the detection of sickle-cell mutation [25] and β-thalassaemia using either SSCP or DGGE to separate the fragments.

b) Detection of gene deletions
Deletion of one or a few base pairs in a gene reduces its length. In the most commonly encountered mutation producing cystic fibrosis, three base pairs are deleted at codon 508. When the cystic fibrosis transmembrane conductance regulatory gene is subjected to amplification around codon 508, one 98 bp fragment is produced in a normal person. In carriers, 98 bp and 95 bp fragments are produced, while in a cystic fibrosis patient one fragment with 95 bp (indicating the loss of 3 bp) is produced. This is a fast and confirmatory test for cystic fibrosis [*26*].

In growth hormone (GH) gene deletion, when the primers for positions flanking both sides of the GH gene are used, no PCR amplification occurs due to the gene deletion. This is a fast and inexpensive test for screening for GH deficiency.

c) Detection of gene rearrangements
Characteristic PCR products are produced in cases with a gene rearrangement and are used to identify chromosomal and gene rearrangements.

d) Detection of chromosome number or fragments
A chromosome-specific primer is used to amplify available chromosomes. If the chromosome concerned is present, then amplification occurs; otherwise no amplification takes place. This is seen in patients with Turner syndrome with a Y chromosome fragment. A Y-chromosome-specific oligonucleotide primer is used and the amplified Y chromosome can be seen following electrophoresis. This technique is also used for fetal sex determination using amniotic fluid or chorionic villus DNA [*10*].

Indirect detection of the mutated gene

For many inherited disorders the basic defect and the gene involved are not known. In these cases, the presence of a defective gene is identified by linkage analysis, a technique that depends on the presence of a specific marker (e.g. DNA polymorphism) in linkage with the abnormal gene.

Linkage studies using either variable number of tandem repeats or restriction fragment length polymorphisms have been used to identify a large number of abnormal genes which lead to disability [*7–9*]. The most common are haemophilia A, cystic fibrosis, adult polycystic kidney disease, carbamoyl phosphate synthetase deficien-

cy, Waardenburg syndrome and Alport syndrome. These diseases can be identified in carriers during prenatal diagnosis or in neonates and later in life by linkage analysis. An abnormal fetus could be identified during prenatal diagnosis using the pattern of the DNA markers in the parents and affected and normal siblings [*27–30*].

Molecular diagnosis of disability in Riyadh

A molecular biology laboratory was established in the 1980s at the Department of Medical Biochemistry, the Referral and Consulting Unit and the WHO Collaborating Centre for Haemoglobinopathies, Thalassaemias and Enzymopathies. Analysis can be done by restriction fragment length polymorphism studies, Southern blotting, SSCP, DGGE, dot blot analysis, reverse dot blot and DNA sequencing. Cytogenetic studies are conducted on karyotype chromosomes, and a fluorescence *in situ* hybridization procedure is used for the detection of chromosomal anomalies. PCR is a frequently used procedure to amplify DNA, and the above-mentioned procedures are employed for mutation detection. These studies are conducted for carrier detection at prenatal, neonatal and postnatal stages as a means of disability prevention.

Future perspective

Since the first report of the use of DNA techniques to identify the sickle-cell gene in 1978, tremendous progress has been achieved in the field of genetic screening and genetic diagnosis. Early detection of a disease state or detection of a carrier state, followed by genetic counselling are essential in control and prevention endeavours.

The goal of genetic diagnosis is the identification of a disease-producing mutation in any family. The initiation of the Human Genome Project has advanced gene(s) identification; genes responsible for disease have been cloned, mutations have been identified and highly accurate testing methods have become available. As carrier screening is possible for many diseases, particularly autosomal recessive disorders, it should be introduced in routine medical practice in order to: (i) arrive at a definitive and accurate diagnosis based on molecular pathology; and (ii) allow genetic counselling and hence accelerate primary prevention strategies.

References

1. La Plante MP. The demography of disability. In: West J, ed. *The Americans with Disabilities Act: from policy to practice.* New York, Millbank Memorial Fund, 1991:55–77.
2. El-Hazmi MAF. The genetic contribution to malformation and handicaps. In: *Proceedings of the symposium on the medical genetics in the setting of Middle Eastern populations.* Riyadh, KACST Press, 1995:72–7.
3. Kalter H, Warkany J. Congenital malformations (Part 1). Etiologic factors and their role in prevention. *New England journal of medicine*, 1983, 308:424–31.
4. Kalter H, Warkany J. Congenital malformations (Part 2). *New England journal of medicine*, 1983, 308:491–7.

5. Culting GR, Antonarakis SE. Prenatal diagnosis and carrier detection by DNA analysis. *Pediatrics in review*, 1992, 13:138–43.

6. Antonarakis SE. Diagnosis of genetic disorders at the DNA level. *New England journal of medicine*, 1989, 320:153–63.

7. Antonarakis SE, Phillips JA 3rd, Kazazian HH Jr. Genetic diseases. Diagnosis by restriction endonuclease analysis. *Journal of pediatrics*, 1982, 100:845–56.

8. Phillips JA 3rd. Clinical applications of gene mapping and diagnosis. In: Childs B et al., eds. *Molecular genetics in medicine. Progress in medical genetics*, 1988, 7:68–99.

9. Phillips JA 3rd. Diagnosis at the bedside by gene analysis. *Southern medical journal*, 1990, 83:868–75.

10. Kogan SC et al. An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences: application to hemophilia A. *New England journal of medicine*, 1987, 317:985–90.

11. Phillips JA 3rd. Clinical applications of DNA analysis to medicine. In: *Proceedings of the symposium on the medical genetics in the setting of Middle Eastern populations*. Riyadh. KACST Press, 1995:36–49.

12. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of molecular biology*, 1975, 98:503–17.

13. Phillips JA 3rd. Inherited defects in growth hormone synthesis and action. In: Scriver CR et al., eds. *The metabolic basis of inherited diseases*, 6th ed. New York, McGraw Hill, 1989:1965–83.

14. Orkin SH et al. The molecular basis of α-thalassaemia: frequent occurrence of dysfunctional α-loci detected by restriction endonuclease mapping. *Cell*, 1979, 17: 33–42.

15. Sutherland GR. Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. *Science*, 1977, 197:265–6.

16. Oberle I et al. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science*, 1991, 252:1097–102.

17. Rousseau F et al. Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation. *New England journal of medicine*, 1991, 325:1673–81.

18. Verkerk AJMH et al. Identification of a gene (*FMR*-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, 1991, 65:905–14.

19. Yu S et al. Fragile X genotype characterized by an unstable region of DNA. *Science*. 1991. 252:1179–81

20. Kremer EJ et al. Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. *Science*, 1991, 252. 1711–4.

21. Lakich D et al. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. *Nature genetics*, 1993, 5:236–41.

22. Collins SJ. Breakpoints on chromosomes 9 and 22 in Philadelphia chromosome-positive chronic myelogenous leukemia (CML). *Journal of clinical investigation*, 1986, 78:1392–6.

23. Butler MG et al. A child with 45,X/46,X,del(Y)(q12) identified with a Y-specific probe. *Fertility and sterility*, 1986, 46:718–20.

24. Saiki RK et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 1988, 239: 487–91.

25. Posey YF et al. Prenatal diagnosis of sickle-cell anemia: hemoglobin electrophoresis versus DNA analysis. *American*

journal of clinical pathology, 1989, 92:347–51.

26. Campbell PW et al. Cystic fibrosis: relationship between clinical status and F508 deletion. *Journal of pediatrics*, 1991, 18:239–41.

27. Antonarakis SE. The molecular genetics of hemophilia A and B in man: factor VIII and factor IX deficiency. *Advances in human genetics*, 1988, 17:27–59.

28. Reeders ST et al. A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. *Nature*, 1985, 317:542–4.

29. Gitschier J et al. Genetic mapping and diagnosis of haemophilia A achieved through a Bcl I polymorphism in the factor VIII gene. *Nature*, 1985, 314:738–40.

30. Germino GG et al. Identification of a locus which shows no genetic recombination with the autosomal dominant polycystic kidney disease gene on chromosome 16. *American journal of human genetics*, 1990, 46:925–33.