GENETIC DISEASE UPDATE – SINGLE GENE DEFECTS

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Contents
Introduction
Technological Advances in Diagnostics
Principles in the Screening and Diagnosis of Common, Single Gene Defects
Thalassaemia
Screening for carrier status
  Antenatal Diagnosis
Therapeutics in Genetic Disorders
Holistic Approach to Genetic Diseases
  Support Groups
Combined Specialist Clinics
Conclusion
INTRODUCTION

Genes are the fundamental units that control protein production in a cell. These proteins are vital to the normal function of a cell e.g. enzymes, structural proteins. Hence a change in a gene that results in either a qualitative or quantitative defect in protein production is likely to result in a disease state. Such changes are known as mutations and the aim of genetic testing is to detect/identify them.

Advances in biotechnology have revolutionised the way we diagnose and treat genetic disorders. The major advances have been in single gene defects. This article looks briefly at some of these advances with particular reference to diagnostic and therapeutic options that are being developed, or are already available.

TECHNOLOGICAL ADVANCES IN DIAGNOSTICS

Although many genetic diseases can be recognized through a combination of pattern recognition and common laboratory tests, making a genetic diagnosis at the molecular level is essential if carrier detection, presymptomatic diagnosis or antenatal diagnosis is to be considered. Furthermore, the choice in therapeutics may be influenced also by the molecular pathology.
Figure 1. Approach to genetic diagnosis

Phenotype

Pattern recognition
+/-
Laboratory tests

Clinical diagnosis

Single gene defect?

Yes

DNA analysis methods

Gene known

Mutation analysis

Mutation known

Indirect testing by linkage analysis

Gene unknown

Mutation analysis

Mutation known

Direct testing by mutation scanning

Mutation unknown

Direct testing by looking for specific mutations

No

Diagnosis may still be possible by
- imaging
- chromosomal analysis
- biochemical tests
The choice of the DNA analysis method used to detect mutations is greatly dependent on whether the gene or mutations causing the disease are known.

1. Detection of unknown gene(s) using linkage analysis

Almost 4000 inherited diseases are caused by unknown mutations in yet undiscovered genes. In some instances, indirect DNA diagnosis can be made using linkage analysis. This involves the use of genetics markers and analysing to see if any of these markers appear to "tag along" with the disease. This method requires many individuals to be tested before a pattern may be discerned. The markers used may not be sited within the target gene hence accuracy is limited.

2. Detection of known and unknown mutations in known gene(s)

A. Detection of known mutations

If the gene associated with a disease is known, mutational analysis is then possible. In some diseases, a few common mutations account for the bulk of the cases. For example, in beta thalassaemia, 4 mutations of the beta globin gene make up 90% of the mutations in our population (Fig.2). Testing for these known mutations first may be more cost effective. These tests are highly accurate but will only detect mutations that are tested for. Hence a negative test does not necessarily exclude a genetic disease.

Methods commonly used include:

i. Southern blot: This is a method that combines gel electrophoresis with the use of specific DNA labelled probes that recognise the abnormal/disease sequence and bind to complementary DNA sequences. This method requires large amounts of DNA, is labour intensive and may take 1-2 weeks to perform. As such, PCR has superceded this method in many labs.

ii. Polymerase chain Reaction (PCR): PCR is a powerful technique that allows for selective, accurate and rapid amplification of target/abnormal DNA sequences. Its advantages are that only very small amounts of DNA are required and results can be obtained in a few days. The disadvantage is that information on the normal DNA sequence must already be known. Many of the examples listed in Table 1 utilise PCR techniques for diagnosis. PCR has also been adapted to detect single nucleotide differences (ie mutations), and this method of detecting a specific pathogenic mutation is known as ARMS (amplification refractory mutation system). This technique is routinely used for the known mutations in thalassaemia.

iii. Allele-specific oligonucleotide (ASO) probe analysis: An extension of the PCR method described above, is the use of two pairs of synthetic probes (one for the normal gene and the other for a specific mutation) as hybridisation probes on DNA that are selectively amplified by PCR. In this way, every sample amplified will be probed against the normal and mutant sequences. Hence the individual
can be fully characterised as homozygote for the mutation (diseased), heterozygote (carrier) or normal. This method will only detect the mutation tested for e.g. thalassaemia.

B. Methods used to screen for unknown mutations (Mutation Scanning)
If the gene affected is known but mutation is still undefined, mutation detection would involve methods such as:

- **Electrophoresis of single stranded/denatured DNA**: Under appropriate conditions, differences in DNA sequences will result in different migration patterns on gel electrophoresis. This method only detects an abnormal DNA segment but does not define the exact mutation, which will require DNA sequencing.
- **DNA Sequencing**: This is the most precise way to characterise a segment of DNA. However, it is not cost effective to be used as a routine method, but is commonly used in research.

These methods will detect changes in the genes, not only the disease genes but some of these may be normal variants (polymorphisms). Differentiating this will require further characterisation of the gene’s function i.e. protein production and protein structure.

| Table 1: Single gene defects that are currently tested for locally by mutational analysis. |
|-----------------|------------------|-----------------|------------------|
| **Disease**     | **Gene(s) affected** | **Comments**                | **Importance**               |
| Thalassemias    | Alpha and beta globin genes | Thalassaemia is the most common single defect in Singapore Single cell PCR is being developed locally for pre-implantation diagnosis | Making a genetic diagnosis at the molecular level is essential if carrier detection or antenatal diagnosis is to be considered. |
| Duchenne Muscular Dystrophy | Dystrophin | Confirmation of carrier status important as 2/3 of the mothers are carriers | |
| Haemophilia A | Gene for Factor VIII | Early prenatal diagnosis important as interventive therapy can be administered antenatally | |
| Congenital adrenal hyperplasia (21-a hydroxylase deficiency) | Adrenal microsomal cytochrome P450 (CYP1) gene | | |

Bulletin 8; September 1998
<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene(s) affected</th>
<th>Comments</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-linked congenital adrenal hypoplasia</td>
<td>DAX-1 gene for adrenal development</td>
<td></td>
<td></td>
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<tr>
<td>Pseudohypo-parathyroidism (type Ia)</td>
<td>Gsa gene for hormone signal transduction</td>
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<tr>
<td>Androgen insensitivity</td>
<td>Androgen receptor gene</td>
<td></td>
<td></td>
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<tr>
<td>Retinoblastoma</td>
<td>Retinoblastoma gene RB1</td>
<td>Useful in identifying hereditary form of retinoblastoma</td>
<td>Increased risk of bilateral, multifocal retinoblastoma in the hereditary form</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>Immunoglobulin chains; oncogenes</td>
<td>For monitoring residual disease and to detect early relapse</td>
<td>Effective as it is very sensitive and will detect the presence of very small amounts of abnormal DNA.</td>
</tr>
</tbody>
</table>

**PRINCIPLES IN THE SCREENING AND DIAGNOSIS OF COMMON, SINGLE GENE DEFECTS**

In common diseases caused by single gene defects, the aims are to
- screen for the carrier status
- provide antenatal diagnosis for a severe disorder
These principles have been successfully illustrated by thalassaemia carrier screening and antenatal diagnosis.

**THALASSAEMIA**

**Screening for carrier status**
In Singapore, the commonest single gene defect is that of thalassaemia. 3% of the population are carriers and hence, are at risk of producing a child with thalassaemia major, a disease with severe morbidity and mortality. Screening is thus of vital importance in prevention. While specific testing and counseling are usually carried out at tertiary institutions, the family physician (FP) plays a vital role in screening for carrier status.

1. Early (pre-conception) screening for thalassaemia trait should be encouraged by the FP who is in contact with young couples planning their families.
2. Noticing a low mean corpuscular volume (MCV) on a full blood count. **This is an important and useful clue which tends to be overlooked** and should alert the FP to the need for thalassaemia screening. The normal range of MCV changes with the age of the child. A good rule-of-thumb is:
   - the lower limit of MCV = 70 + age of child (in years)
Thus, if a 3-year-old child has an MCV of < 73, this is regarded as microcytosis, and investigations would be necessary to ascertain the cause. In general, for the same degree of anaemia, patients with β-thalassaemia trait have a lower MCV, usually < 70 (and also a higher RBC count) compared to those with iron deficiency. The MCV values seen in alpha thalassaemia is more variable. Thus, if we see a 5-year-old child with a Hb level of 10 g/dl, RBC count of > 5 x 10^{12}/L, and an MCV of 65 fL, we can be almost certain that the diagnosis is thalassaemia trait rather than iron deficiency (see Fig 2: Mentzer’s index.).

**Figure 2. Approach to Person with Microcytosis**

![Diagram of approach to person with microcytosis]

- **Person with low MCV on routine FBC**
  - Mentzer’s index (MCT/RBC)
  - PBF: target cells
    - >13: Probably Fe-deficient
    - <13: Probably thalassaemia trait
  - Check for Fe-deficiency
    - Fe <7 μmol/L
    - Ferritin <20 μg/L
    - Transferrin >300 mg/dL
  - Fe deficiency
    - Investigate cause
    - Correct deficiency
  - Thalassaemia masked by Fe-deficiency.
    - If microcytosis persists, after normalization of Fe status
  - Stain for HbH inclusions
  - Glycerol lysis resistant & Alkali resistant Hb*
    - HbA2 >3.5%
    - HbH disease: 3 gene deletion
  - α-thalassaemia trait
    - α2 trait: 1 gene deletion
    - α1 trait: 2 gene deletion
  - β-thalassaemia trait

*Alkali resistant Hb reflects the presence of HbF which should be absent beyond the age of 1 year.

Bulletin 8; September 1998
Antenatal Diagnosis for Thalassaemia

Only when carriers have been identified, can genetic counseling follow suit. Options of prenatal diagnosis are only offered to parents at risk of producing a Barts Hydrops or Beta thalassaemia major as these conditions carry high morbidity and mortality. For at risk couples who choose to have antenatal diagnosis, it is important to be able to identify the mutations in the parents as this determines the interpretation of the antenatal diagnosis.

Fig 3. Prenatal Diagnosis for Thalassaemia

Both parents are phenotypically carriers of the same thalassaemia trait

α trait

β trait

Confirm parents’ genotype

by PCR or sequencing

Both parents have SEA deletion*

Both parents have β mutations

>90% of mutant alleles in our local population are found in

• IVS-II-654
• Codon 41/42
• Codon 17
• Codon 28

Risk of Bart’s Hydrops is 1 in 4

Risk of β-thalassaemia major is 1 in 4

Advise prenatal diagnosis

Chorionic villus sampling at about 10 weeks gestation

PCR genotyping (takes 2-3 days)

Confirm presence/absence of Bart’s hydrops

Confirm presence/absence of β-globin mutations
*The commonest α-thalassaemia mutation encountered locally is the South-East Asian (SEA) deletion, the result of the loss of 2 Alpha-alleles in cis-position (ie, on the same chromosome).

#For β-thalassaemia, molecular diagnosis is more difficult as there exists many different mutations. However, the most common mutations in each of the three ethnic groups have been identified, and a quick screen can be made for these mutations. In cases where the screen is inconclusive, confirmation of the specific genotype is sought by a combination of methods including analysis of globin chains, reverse dot blot using ASO, and DNA sequencing.

**Figure 4**

4a: PCR Diagnosis of Barts hydrops
Gel shows results of 2 separate PCRs assays. Presence of upper band in samples from father, mother and foetus denotes presence of SEA deletion. Absence of lower band in sample from foetus denotes absence of α-globin genes, hence this is a Barts hydrops.

4b: PCR Diagnosis of β-thalassaemia major
Gel shows results of multiplex PCR with primers for 2 different mutations, as well as internal control. The father is a carrier for the IVS1 #5 mutation while the mother is a carrier for the the codon 41-42 mutation. The foetus is a compound heterozygote with both these mutations and is therefore a β-thalassaemia major.
THERAPEUTICS IN GENETIC DISORDERS

Despite the identification of the genetic defect in many single gene diseases, gene-specific therapies have yet to be formulated for clinical use in most cases. Nevertheless, various advances have been made in therapeutics that help to improve the quality of life. Some of these are palliative while others are curative. Examples of genetic conditions for which newer therapeutic options are available locally are summarised in this table.

Table 2: Examples of Treatment Available locally for Genetic Disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>What’s New</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Turner Syndrome</td>
<td>Recombinant growth hormone</td>
<td>Effective in overcoming problem of short stature</td>
</tr>
<tr>
<td>Storage diseases eg glycogen storage disease</td>
<td>Liver transplant for glycogen storage disease</td>
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<tr>
<td>Osteogenesis imperfecta</td>
<td>Pamidronate infusion (Bisphosphonates)</td>
<td>Increases bone mineral density</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clinical trials in progress, preliminary results encouraging</td>
</tr>
<tr>
<td>Thalassaemia</td>
<td>Parenteral Iron chelator therapy, Frequent and home administered Bone marrow transplant</td>
<td>Chelators reduce morbidity from iron overload but do not cure disease. Compliance important in efficacy of treatment Curative</td>
</tr>
</tbody>
</table>

HOLISTIC APPROACH TO THE MANAGEMENT OF GENETIC DISEASES

Genetic diseases tend to present with multiple problems, which are often chronic as well. To help parents and patients cope with these problems, support groups and special societies have been formed in Singapore.

These include the Down Syndrome Association, the Haemophilia Society, the Turner Syndrome Support Group, the Rainbow Club and the Transplant Support Group. Such support groups seek to improve understanding of the unique problems of each condition eg the recently-formed Turner Syndrome Support Group organises activities attended by parents and patients where they can meet with paediatric endocrinologists and cardiologists, geneticists, child psychologist and social worker.
Children with genetic diseases resulting in multiple problems also benefit from combined specialist clinics as which they are seen jointly by doctors and other therapists from different specialties. An example of this is the Bone Clinic, where patients with skeletal dysplasias are managed jointly by paediatric orthopaedic surgeon, paediatric endocrinologist, geneticist and physiotherapist. Another example is the Rehabilitation Clinic where patients with muscular dystrophies and spinal muscular atrophy are jointly managed by the neurologist, orthopaedic surgeon, pulmonologist, physiotherapist and occupational therapist.

CONCLUSION

In Singapore, antenatal screening for thalassaemia has succeeded in dramatically reducing the number of livebirths with thalassaemia major. However, for patients and families with genetic disorders, the burden of the disease continues to be a heavy one. While the management for such patients often requires multidisciplinary specialist care, there remains an important role for the FP in the holistic management of the patient and family.