1.
The Current Practice of Genetic Testing

Chris Scerri

The completion of the draft sequencing of the Human Genome has opened up new horizons in the diagnosis, prevention and treatment of genetic disease. The genome of an organism constitutes the whole "blueprint" where all the instructions for all the functional proteins of the organism are stored. The human genome is divided into 22 pairs of autosomal chromosomes together with two X chromosomes in females and an X and a Y chromosomes in males. The genetic compliment is situated within the nucleus of each cell of the body. The chromosomes themselves can be further divided into individual genes that code for individual proteins. The genes (and thus the chromosomes) consist of tightly coiled threads of deoxyribonucleic acid (DNA). DNA is itself formed of smaller subunits made up of one of four nitrogenous bases (adenine, guanine, thymine and cytosine), covalently attached to a deoxyribose moiety and a phosphate group. The bases are covalently linked through phosphodiester bonds between the carbohydrate groups. In addition, two strands of DNA polymer, bind to each other through hydrogen bonds between the nitrogenous bases. This arrangement is analogous to a ladder where the upright sides are represented by the carbohydrates linked by phosphodiester bonds whilst the bases linked through the hydrogen bonds are analogous to the rungs of the ladder. The binding of the bases by the hydrogen bonds is not haphazard but definite pairs of bases always bind together; thus adenine always binds with thymine whilst guanine will always bind with cytosine. This complimentarity forms the basis of both cell division and replication as well as protein formation.

During meiosis one copy of each chromosome pair segregates in the individual oocyte or sperm. This effectively means that each oocyte or sperm has half the compliment of chromosomes (23 and the X or Y). Thus, following fertilisation, the proper compliment of chromosomes is assured. During mitosis, the DNA strand unwinds and each single strand acts as a template for a new, complimentary strand. Thus each daughter cell receives a strand derived from the mother cell and a new complimentary DNA strand. Thus whilst meiosis ensures that the embryo has a normal compliment of chromosomes and that it receives half its compliment from the mother and the other half from the father, mitosis ensures that all the cells of the body contain identical copies of all the chromosomes.

Human Genes

In general, a gene can be divided into five general areas:

1. upstream sequences to the gene/s of interest that regulate the transcription of the same gene/s;
2. the initiation codon that signifies the start of translation of the protein;
3. exons, the coding regions, that alternate with the introns, the non-coding sequences, of the genes;
4. the stop codon signifying the end of translation of the protein;
5. downstream sequences that have important regulatory and RNA stabilisation functions.

Any mutations, even single base mutations, in any of these 5 areas, have a potential to disrupt the normal function of the gene. This forms the basis of genetic disorders.

Inheritance

Genetic disorders can be classified into two main groups:
1. genetic disorder that show a Mendelian type of inheritance
2. genetic disorders that show a non-Mendelian type of inheritance

In Mendelian inheritance, the phenotype is dependent on the genotype at a single locus. Examples of Mendelian inheritance include thalassaemia, cystic fibrosis, Duchenne muscular dystrophy and gangliosidosis.

In non-Mendelian inheritance, the phenotype is dependent on at least two genetic loci with greater or lesser contribution from environmental factors. Examples of non-Mendelian inheritance include ischeamic heart disease and cancer.

Genetic Tests

Genetic tests involve the identification of mutations either within the genes of interest or in their regulatory sequences. This is done by scanning the individual's DNA sequence for possible mutations. The indications for genetic tests include:
- carrier screening,
- newborn screening;
- presymptomatic testing for predicting adult-onset disorders such as Huntington's disease;
- presymptomatic testing for estimating the risk of developing adult-onset cancers and Alzheimer's disease;
- confirmational diagnosis of a symptomatic individual;
- forensic/identity testing;
- and where acceptable prenatal diagnostic testing.

For these tests, the DNA can be obtained from any nucleated cells though in general the sample is either obtained from blood or from internal cheek cells (mouth wash sample).

The testing strategies

One has to understand that though we speak of a genetic test, the testing strategy can differ between genetic disorders and in most cases can be very time consuming. In the case of a disorder characterised by Mendellian inheritance and the gene is known, the initial step following DNA extraction is that of gene amplification. Gene amplification is the term used when the gene or parts of, are selectively amplified in-vitro. This is done by adding a pair of synthesised, short, oligonucleotide strands (primers) that flank the area of interest. One of the primers is complimentary to one strand whilst the other primer is complimentary to the opposite strand. Together with these primers, nucleotides, an appropriate buffer and a thermostable DNA polymerase are added. The whole solution is then passed through repeated cycles of heating to 95°C (denaturing), cooling to a temperature usually ranging between 55°C and 65°C (annealing of the primers) followed by heating at 72°C (elongation of the primers by the DNA polymerase). This set of temperature changes are cycled up to 30-35 times. At the end of these cycles, the area flanked by the two primers would have been preferentially amplified in an exponential manner i.e. if one starts with one copy theoretically one would end with 2^{35} copies of the area of interest. This is called the polymerase chain reaction or PCR.

Following PCR, the fragment can be sequenced directly and the sequence compared to the normal sequence, or else allele hybridisation-based techniques can be used to probe the gene of interest by short oligonucleotide probes complimentary to either the normal strand or the mutated strand. These techniques can be utilised in the service-oriented setup either if the gene is small (2000-4000 base pairs) or if the prevalent mutations within the community are known and their number is small (8-10). If the gene is large and the prevalent mutations are not known, then sequencing becomes impractical due to

cost (one sequencing run can read up to 700 bp) and at the same time allele hybridisation-based techniques cannot be used. In such cases, one enters the realm of research, as techniques (e.g. single strand conformational polymorphism, SSCP) are utilised to identify possible areas within the gene that are mutated following which sequencing is performed on the identified areas.

If the disorder is characterised by Mendelian inheritance, the gene is known but most of the mutations are large deletions, then the technique that is normally utilised is one of restriction enzyme digest followed by hybridisation to labelled probes. Restriction enzymes are bacterial enzymes that digest DNA at particular DNA motifs. Thus, once total genomic DNA is digested by restriction enzymes, the resultant DNA is fragmented into specific fragments of particular size depending on the spacing of the particular motifs in the DNA. These DNA fragments can be separated in a gel depending on size and then blotted on to a membrane. Once blotted, the DNA fragments are transferred on to the membrane. The blotted DNA fragments are then hybridised with a labelled probe complimentary to the gene of interest. Depending on the size of fragment to which the probe has hybridised, one can deduce if a large deletion has occurred in or very near to the gene of interest. As one can already imagine the procedure is quite time consuming, can be difficult to interpret and due to the fact that the best results are obtained by radiolabelled probes, there is the problem of radioactive waste.

In the case of a disorder characterised by non-Mendelian inheritance but some or all of the involved genes are known, then a risk assessment can be achieved by one or all of the above mentioned techniques. On the other hand if the disorder is characterised by either Mendelian inheritance or non-Mendelian inheritance and the gene/s is/are not known, then a risk assessment can be made by an extensive family

examination through the utilisation of single nucleotide polymorphism (SNP's, sometimes called markers) can be obtained. In this way, with the help of particular SNP's that are co-inherited with the disorder a predictive risk assessment for an apparently unaffected individual can be produced.

Conclusion

It should be clear that this short discussion of genetics and the major testing strategies utilised in the identification of genetic disease does not cover all the possible testing strategies. In addition, testing strategies do not only depend on the particular disorder or gene but also on the amount of basic research done towards the identification of the prevalent mutations within an ethnic community or region. Thus it should be emphasised that prior to the setting up of genetic testing for a particular disease, a properly conducted genotyping research study on the particular disorder should be undertaken. Unfortunately, in Malta this has only been accomplished in thalassaemia, which up to this date is the only genetic disease in Malta that is fully characterised. In addition, some information has been gathered on the prevalent mutations for cystic fibrosis, ganglioasidosis and dihydropteridine reductase deficiency (DHPR or atypical phenylketonuria). Though considering our size and financial constraints, these results can be described as relatively big leaps, one hopes that further attention and funding be committed especially in the field of the polygenic disorders such as familial cancer, heart disease, asthma and diabetes.

Genetic tests seem to confer the ability to diagnose genetic disease with relative ease but one has to keep in mind that these are very particular and special tests. It should be remembered that the result of a genetic test, especially in predictive or carrier testing, can be very devastating to the patient, could result in stigmatisation, and can involve the

family as a whole. It should be clearly emphasised that these tests should be carried out only after full and accurate information is offered to the client together with pre- and post-testing counselling. Proper and informed consent - the emphasis being on the word informed - has to be obtained from the client and some would even argue, from the family. It should thus be obvious that such information and testing should preferably be done through a properly organised clinic that apart from the clinical specialists should also include counsellors and psychologists. The client should be made to feel that he has all the support that is necessary, that this support can be given at all times, and that he can obtain the most recent and up-to-date information. To quote from the first newsletter of the Bioethics Consultative Committee, "it would be a great pity if it was misused as many screening tests have been misused in the past."