The Insulin Gene and Diabetes Mellitus

A New Approach

Diabetes mellitus comprises a heterogeneous group of disorders characterized by chronic hyperglycaemia, and a propensity to develop microangiopathy, neuropathy, nephropathy and atherosclerosis. It is a common condition and is seen in all ethnic groups. The causes of diabetes are poorly understood, but appear to involve some form of interaction between hereditary and environmental factors.

The genetics of diabetes is still unclear, but a picture is slowly emerging. Recently, associations with two HLA-DR antigens 3 and 4 (coded for by genes on chromosome 6) have been demonstrated with insulin-dependent diabetes, but are not sufficient to explain the entire genetic component of this disease. It has been postulated that a second gene locus might be involved. One such gene may be the human insulin gene which is located on the short arm of chromosome 11.

Direct Gene Analysis

The last 10 years or so has seen the emergence of a new technology called Recombinant DNA analysis which has enabled the scientist to study inherited disorders at a DNA level. Not only have these techniques afforded the ability to analyse variations in DNA in man but they have been put to commercial use in the invaluable production of several hormones and vaccines such as human insulin itself, growth hormone and interferon.

The procedures that are used are somewhat complex, but the thumbnail sketch of these strategies and their application to inherited disorders such as diabetes now follows.

It is known that DNA in chromosomes — the 'genome' — is double-stranded with one strand having a nucleotide base sequence complementary to the other. This is by virtue of the fact that the nitrogenous...
base adenine only pairs with thymine and guanine only
pairs with cytosine. Now, the total amount of DNA in
a single human cell is about 6x10^9 bases long, whereas
a single gene, on average, is about 1,000 bases in
length. There are probably only about 50,000 genes in
the human genome and therefore a large proportion
of the genetic material, roughly 95% does not code for
proteins. Nevertheless, to isolate just one of these
genes seems a formidable task. This is not so, how-
ever. Using a special enzyme called reverse
transcriptase, a DNA copy (cDNA) of the messenger
RNA of a particular gene can be synthesized under
certain laboratory conditions. Messenger RNA can be
relatively easily isolated if the amino acid sequence of
the protein (in our case insulin) is already known. The
cDNA can then be radioactively labelled by adding
radioactive bases and hence used as a 'probe' to look
for complementary sequences in genomic DNA, i.e.
to search out and 'light up' the gene under study to the
exclusion of all other genes. The next requirement
now is to make sufficient copies of this DNA fragment
to be able to handle it in the laboratory. This 'cloning'
is achieved by inserting this DNA fragment into the
DNA of a rapidly replicating host such as E. coli. This
cloned DNA can be made single-stranded and then
labelled with radioisotope; it is now called a 'DNA
probe' because it is capable of locating and
hybridizing with DNA of any individual that carries
the complementary sequence. It is quite easy to make
DNA from the nuclei of white cells obtained from a
peripheral blood sample. However, because the size
of the DNA probe is so much smaller than the
genomic DNA, the latter can be cut up into millions of
fragments by digestion with other enzymes known as
restriction enzymes. These enzymes, produced from
bacteria, always cleave DNA at a small number of
reproducible sites. The fragments so formed can be
roughly separated according to their size by
electrophoresis and then, while separated, the
radioactive DNA probe can be added so that it
hybridizes with the fragment that contains the
complementary sequence. This results in a
radioactive band which can be revealed by making an
autoradiograph.

Now, if the restriction enzyme cuts the DNA at
always the same site, radioactive bands of the same
size are expected. If, for some reason, for instance a
mutation that destroys this cutting site or creates a
new one, or if an extra bit of DNA (an 'insertion') is
found within the cutting sites, then different sized
radioactive bands are to be expected. Such variations
in DNA sequence, if present in more than 2% of
the population, are called polymorphisms. These
polymorphisms are fairly common in the human
genome; they may be found within a gene or in a bit of
DNA that does not code for protein. They may be
harmless, yet if found within the gene or sufficiently
close to it, then they may affect the expression of the
gene and thus result in a disease. A typical example is
sickle cell disease where a polymorphism found within
the β-globin gene results in the substitution of thymine
by adenine in the 6th amino acid of the gene. The most
important feature of a polymorphism is its ability to be
transmitted through families in a simple Mendelian
fashion, and therefore, if associated with a disease,
then this disease can be tracked through future
generations.

The Insulin Gene

In their studies of the human insulin gene, Professor Bell and his colleagues in San Francisco
were the first to discover a polymorphism just outside
this gene. With the use of an insulin probe and the
techniques outlined above they observed a highly
variable stretch of DNA which was broadly divided
into a 'short' DNA insertion approximately 600 bases
long and a 'long' insertion of over 2,000 bases long
(Fig. 1). Individuals can inherit the same insertion from
both parents (hence homozygotes), thus producing
one band only on an autoradiogram, whereas
heterozygotes will inherit both and display two bands
(Fig. 2). Because of the close proximity of this
polymorphism to the insulin gene, it was soon
postulated that it might play an aetiological role in
diabetes. This hypothesis was strengthened by the
knowledge of mutant insulins that have resulted in this
disorder. Studies therefore were soon underway to see
an association with the different types of
diabetes. The first study, however, by Bell's group,
revealed no differences between NIDDM and
controls, but these workers noted that less than 10%
of the normal population were homozygous for the
long insert. Since then, however, three independent
studies, including work in our laboratory, have
revealed that subjects homozygous for this insert
were indeed found to have a 4-5 times higher relative
risk of concurrence of NIDDM.

The situation with insulin-dependent diabetes
was even more clear-cut. To date, over 150 such
diabetics have been studied in two centres with the
insulin gene probe and the incidence of homozygosity
for the short insertion was found to be almost double
that in controls.

So, what can one make of these results? Certainly they offer an exciting theory that this
cryptic stretch of DNA flanking the insulin gene
might be playing a causative role in diabetes. Several
questions though have yet to be answered. Is the
polymorphism affecting insulin gene transcription
and thus leading to inappropriate insulin synthesis? Or
could it simply be a genetic marker for another
abnormality, hitherto undiscovered, within the insulin
gene itself? Could this polymorphism have risen by
random genetic drift, and the association with both
types of diabetes be entirely spurious? The results so
far must be considered preliminary because the
number of patients studied are relatively small. The
erlier results could be conflicting because of sample
bias and racial differences. In support of the latter
theory, we have studied subjects from different racial groups and observed several differences in non-Caucasian individuals, particularly in an intermediate sized insertion (1,000 bases) seen only in the Negroid race. Up to now, therefore, this polymorphism can only be used in studies on the Caucasian segment. We have also attempted to see if this insulin polymorphism could be responsible for the appearance of impaired glucose tolerance in other conditions, such as gestational diabetes, acromegaly and Type III hyperlipidaemia, but results have been conflicting.

Clearly, therefore, further work is required. The impact of these recombinant DNA techniques into modern medicine, however, is undisputed. For the first time, the ability to study genes themselves has become a practical proposition. Already these techniques have been applied clinically to the antenatal diagnosis of other genetic diseases, such as the haemoglobinopathies, the implications of which are enormous. Future work on the polymorphism flanking the insulin gene should hopefully elucidate the aetiology of diabetes, or at least offer a better guide to classification and understanding of this group of disorders. The next decade should see many more discoveries, and the real possibility now exists that faulty genes responsible for human disease might be replaced.

References: