The Insulin Gene and Diabetes Mellitus

A New Approach

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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

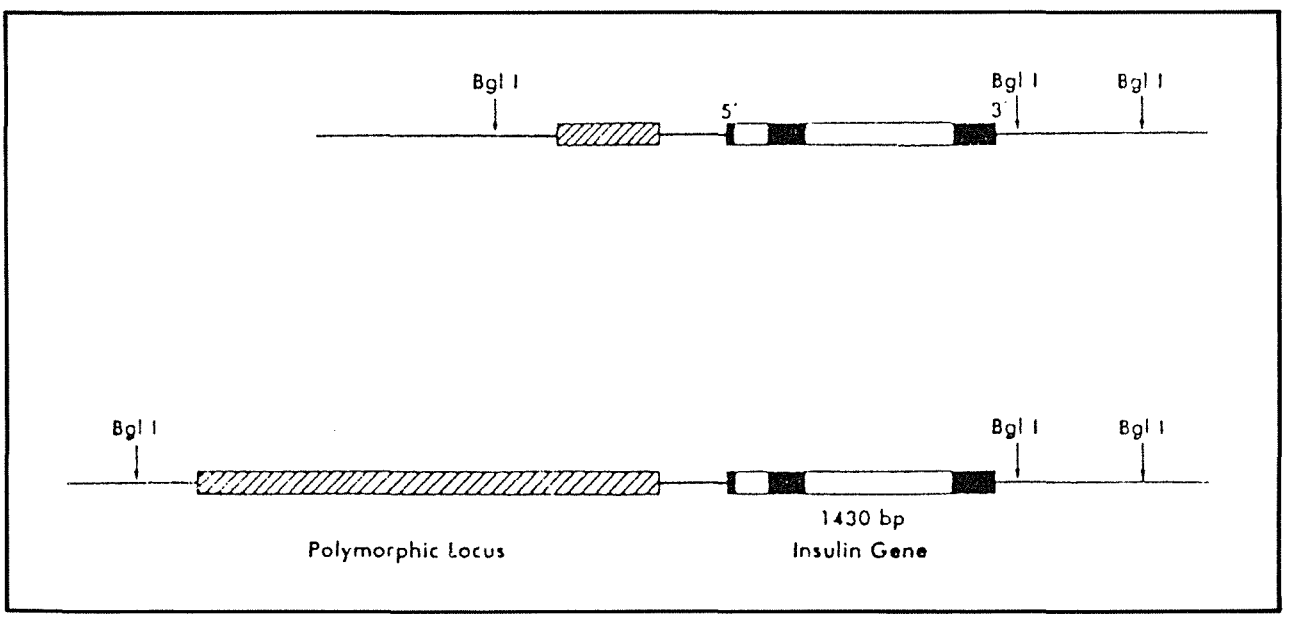

Fig. 1. A diagrammatic map of the human insulin gene illustrating the short DNA insertion (above) and long DNA insertion (below) just before the gene. Bgl 1 is the name of the Restriction Enzyme that cuts the DNA at the sites indicated, resulting in fragments that when 'hybridized' with the insulin probe result in radioactive bands, as seen in Figure 2.

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 6×10^9 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, however. 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 ß-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.[2] 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.[3] Studies therefore were soon underway to seek an association with the different types of diabetes. The first study, however, by Bell's group, revealed no differences between NIDD's and controls, but these workers noted that less than 10% of the normal population were homozygous for the long insert.[2] 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 NIDD[4-6].

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.[7,8]

So, what can one make of these results? Certainly they offer an exciting theory that this polymorphic 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 earlier results could be conflicting because of sample bias and racial differences. In support of the latter

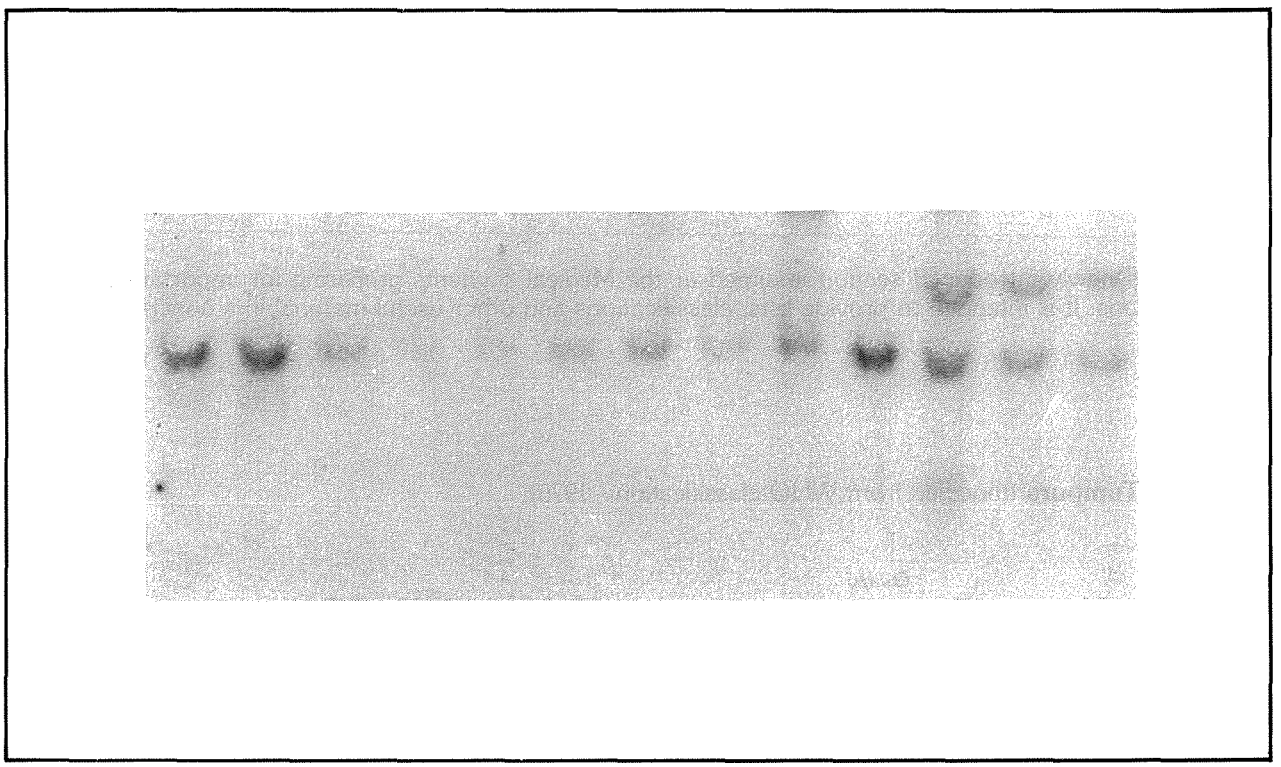

Fig. 2 This is an autoradiogram of DNA from different individuals hybridized with the insulin probe. The top band represents the large 'insertion', whereas the bottom band represents the short 'insertion'.

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.[9] 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,[10] 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.

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