MOLECULAR BIOLOGY OF NON-INSULIN-DEPENDENT DIABETES MELLITUS (NIDDM)

G.R. COLLIER and K. WALDER

Summary

Scientific evidence supports a genetic susceptibility to NIDDM which, in the presence of certain environmental triggers, culminates in the disease phenotype. A number of approaches have been used in attempts to discover what these predisposing genes are, including 'candidate gene' and positional cloning studies, without significant success to date. Because of the heterogeneity and late age of onset of NIDDM, animal models such as *Psammomys obesus* (Israeli sand rat) and new molecular biology techniques may provide the best opportunity yet to discover the genetic cause(s) of NIDDM.

I. INTRODUCTION

NIDDM is one of the most common metabolic diseases and represents a major health problem in both developed and underdeveloped countries. Although it is generally accepted that NIDDM is a genetic disease, the relative importance of genetic predisposition and environmental factors are keenly debated in the literature (Hales 1994; McCarthy et al. 1994). Following many years of research, basic issues such as mode of inheritance, number of loci involved and pathogenesis of the disease remain significant future scientific challenges. Major efforts worldwide are now concentrated on a search for 'diabetes genes' and results of these studies are certain to improve our understanding of the physiological events leading to NIDDM and development of more effective therapies.

II. EVIDENCE THAT NIDDM IS A GENETIC DISEASE

The evidence supporting a genetic susceptibility to NIDDM can be broadly divided into twin studies, family studies, and population studies. The study of twins is a powerful tool for elucidating genetic influences in the development of a disease. A number of studies of NIDDM in monozygotic versus dizygotic twins have shown greater concordance in identical twins (Barnett et al. 1981; Newman et al. 1987; Rotter and Rimoin 1987) suggesting a genetic susceptibility to NIDDM. However, the extent of contribution by genetic factors is impossible to determine considering the variability in concordance reported in the literature. The degree of concordance varies from 28 to 91% in monozygotic twins, and is generally much lower than the often-cited concordance rate (approximately 100%) of the early studies in the United Kingdom (Barnett et al. 1981). Two important factors emerge from this variability in concordance. Firstly, the less than 100% concordance may be related to inadequate follow-up in a number of these studies. For example, in a study by Newman et al. (1987) the initial concordance in monozygotic twins was 58%; however after follow-up, most pairs initially discordant became concordant (Newman et al. 1993). This illustrates an inherent problem in genetic studies of NIDDM as the age of onset of the disease varies and is often quite late in life. Secondly, the presence of discordance among monozygotic twins implies that environmental factors, including diet and exercise, may contribute

School of Nutrition and Public Health, Deakin University, Geelong Victoria 3217

significantly to disease development.

In studies examining the familial aggregation of NIDDM the association is striking, but it must be considered that these associations also reflect shared environment as well as genetic effects. Studies examining Pima Indian families in North America have demonstrated that age- and obesity-adjusted incidence of NIDDM was two to three times higher in subjects with one diabetic parent and up to four times higher in subjects with two diabetic parents than in those with two non-diabetic parents (Knowler et al. 1981). The severity of diabetes, insulin resistance and obesity also aggregate into families in Pima Indians (Knowler et al. 1981). In addition, family studies in a number of other populations have confirmed these results and demonstrate an increase in NIDDM in siblings and first degree relatives of diabetic parents compared with non-diabetic parents (Simpson 1964; Simpson 1968; Kobberling 1971; Baird 1973; Keen and Jarrett 1976; Keen et al. 1982; Cheta et al. 1990). Interestingly, an interaction between familial aggregation of insulin sensitivity and the impact of obesity on severity of insulin resistance has been demonstrated. These studies suggested that offspring of diabetic parents became more insulin resistant for every increment in body weight than individuals with no family history of diabetes (Warram et al. 1990).

Further evidence supporting the genetic basis of NIDDM comes from population studies. Within a given environment and adjusted for obesity, it is clear that diabetes is far more prevalent in certain ethnic groups. A high prevalence of NIDDM in South Pacific islanders such as Nauruans and other isolated populations has been extensively reported (Zimmet and O'Dea 1993). Neel (1962) proposed the 'thrifty gene' hypothesis to explain the persistent genotype that results in an adverse phenotype in modern 'westernized' societies. Additional support for the genetic basis of NIDDM is found in population studies examining genetic admixture. Serjeanston et al. (1983) reported that prevalence of NIDDM in Nauruans over 60 years of age was 83% in fullblooded, but only 17% in Nauruans demonstrated to have a genetic admixture. This has been further supported by other genetic admixture studies (Knowler et al. 1988).

III. TECHNIQUES FOR FINDING DIABETES GENES

One of the major difficulties in determining the genes responsible for the development of NIDDM is the intrinsic heterogeneity of the disease. Genetic studies are further complicated by the gaps in our knowledge of the pathophysiology of NIDDM and our ignorance regarding the primary defect. This has resulted in research groups studying populations or families with discrete types of diabetes such as maturity-onset diabetes of the young (MODY). Consequently, no single genetic approach is the 'correct' one and, in fact, two fundamentally different approaches have been used; the 'candidate gene' approach, and positional cloning.

(a) Candidate gene studies

The candidate gene approach examines specific genes that have been highlighted in physiological and biochemical studies of the pathogenesis of NIDDM. The basic principle of these studies involves comparing the frequency of polymorphisms between diabetic and control populations. It is theoretically possible to examine all genes that have been implicated in the control of insulin secretion and insulin action in an attempt to uncover a mutation which may indicate a predisposition to NIDDM. Despite our rapidly growing knowledge regarding the biochemical basis of the pathophysiology of diabetes, the search for mutations in candidate genes has been disappointing. One of the earliest published mutations involved the insulin gene (McCarthy and Hitman 1993). Patients with an insulin gene mutation, however, were not insulin resistant or obese, and over the past ten years only five distinct mutations have been identified making this an extremely rare cause of diabetes. An obvious progression from the insulin gene was to the insulin receptor and recent studies have identified mutations in the insulin receptor gene (Turner et al. 1989). However patients carrying this mutation have a very severe form of insulin resistance with clinical symptoms not common in NIDDM, including leprechaunism. Over the past four years,

more than 20 mutations have been identified in the insulin receptor gene, but less than 1% of 'common' NIDDM patients have these mutations.

Other studies have concentrated on key steps in insulin action including glycogen storage and glucose uptake which are defective in NIDDM. However, despite the apparent importance of glycogen synthase activity in the pathogenesis of NIDDM (Groop et al. 1993b), recent studies utilizing skeletal muscle biopsies from NIDDM patients concluded that mutations of glycogen synthase or its catalytic subunits are not the cause of reduced enzyme activity (Vaxillaire et al. 1994). Similarly, no mutations in glucose transporter genes have been found in NIDDM (Choi et al. 1991; Kusari et al. 1991) and sequencing of the entire Glut4 (insulin-sensitive glucose transporter) gene failed to detect any differences in diabetic patients (Kusari et al. 1991).

Although our understanding of the basic physiology and biochemistry of diabetes is improving, our lack of knowledge regarding the primary defect makes it difficult to use the candidate gene approach effectively.

(b) Positional cloning

The second approach examines markers on human chromosomes in population-based studies. This approach is not limited by the current understanding of the pathophysiology of diabetes and has the ability to uncover novel genes. Most studies examine the segregation of polymorphisms with the disease phenotype and statistically calculate the probability that the polymorphism cosegregates with a nearby diabetes gene, rather than by chance. One of the problems with conducting these studies is obtaining complete multi-generational families because of the late onset of disease and the premature mortality in patients with diabetes. These genetic studies can also utilize molecular screening techniques now available which allow large-scale screening of gene variation in populations. Currently the most common method is to screen candidate loci for mutations using single-strand conformation polymorphism (SSCP) followed by sequencing to identify the molecular basis of any mutation. In addition there are new techniques including subtraction hybridization (Reynet and Kahn 1993) and differential display polymerase chain reaction (ddPCR) (Liang and Pardee 1992) which allow patterns of gene expression in relevant tissues to be analysed in diabetic and control families to identify novel genes of potential relevance to the diabetic state.

One of the early popular methods of positional cloning was linkage analysis. Researchers have examined patients with Maturity Onset Diabetes of the Young (MODY), a small subgroup of diabetics that are easier to study in linkage analysis because of a simple pattern of inhelitance. Studies in groups of MODY have established a link with the glucokinase gene in approximately 50% of families examined (Hattersley et al. 1992). In addition, mutations have been identified in exon 7 of the glucokinase gene in a French family with MODY (van den Ouweland et al. 1992). These were exciting findings and potentially important for all diabetics as glucokinase acts as a glucose sensor in both the pancreas and the liver. However, recent studies in families with the more common variety of NIDDM have excluded a major role for glucokinase (Elbein et al. 1993; 1994). Other linkage analysis studies have utilized a polymorphic area found in the glycogen synthase gene and demonstrated that a subgroup of the Finnish population, identified by a strong family history of diabetes, have an increased frequency of this polymorphism (Groop et al. 1993a). However, further studies in both French and Japanese populations have failed to confirm this linkage (Zouali et al. 1993; Kadowaki et al. 1993). Research groups have also studied the high-risk Pima Indian population, describing a significant linkage of the fatty acid-binding protein — 2 ($FABP_2$) locus of chromosome 4q with in vivo insulin action (Prochazka et al. 1993). The $FABP_2$ locus has been subsequently investigated in three European NIDDM communities (Finnish, English and Welsh) and these studies failed to find any association between allele frequency and glucose intolerance (Humphreys et al. 1994).

As can be seen by this brief summary only a minority of genes have been identified to date. Very few studies have utilized the new techniques of gene screening and future investigations using these methods will undoubtedly uncover a number of novel genes linked with diabetes. One recent study using subtraction cloning identified a novel protein named RAD, later demonstrated to be important in insulin signalling (Reynet and Kahn 1993). RAD has subsequently been shown to

be over-expressed in skeletal muscle of NIDDM subjects compared with non-diabetics (Kahn 1994).

IV. ANIMAL MODELS

As discussed above, determining the key genetic defects in patients with NIDDM is difficult. One of the main impediments to this research is obtaining multi-generational families to study. Because of these problems, researchers have turned to animal models which have the major advantage of developing phenotypic changes such as obesity and diabetes over a much shorter period of time, usually only months. A number of animal models have been useful in genetic studies of diabetes and obesity, including ob/ob mice, obese Zucker rats, db/db mice, and spiny mice. However, the animal model which most closely mimics the development of obesity and NIDDM in susceptible human populations appears to be *Psammomys obesus*, the Israeli sand rat. The development of hyperglycemia in *Psammomys obesus* following ad libitum feeding of laboratory chow was first demonstrated in 1964 (Schmidt-Nielsen and Haines 1964). In conjunction with hyperglycemia, other physiological defects reported include hyperinsulinemia, impaired glucose tolerance, obesity, cataracts, and hyperphagia (Barnett et al. 1994a).

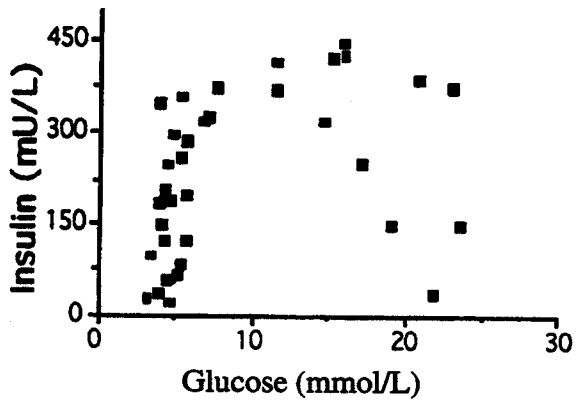

Figure 1. Relationship between fed glucose and insulin levels in *Psammomys obesus*.

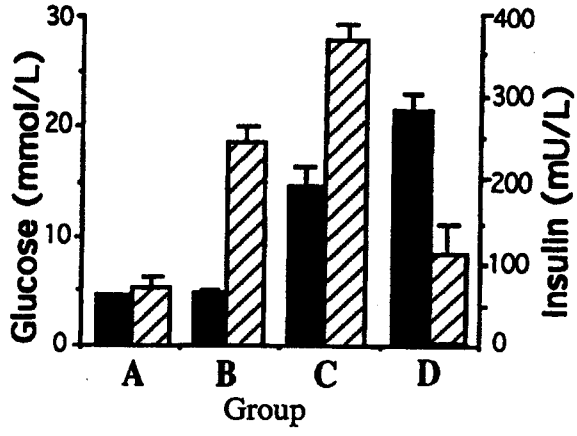

Figure 2. Data for *Psammomys obesus* groups.

In addition, the inverted U-shaped curve has described a relationship between fed glucose and insulin levels in *Psammomys obesus* (Figure 1). This data is similar to that presented in human studies; as the fasting glucose levels increased within the cross-sectional data, there was a progressive increase in insulin levels, and a further increase in glucose levels was associated with a rapid decline in insulin levels. De Fronzo has named this relationship 'Starlings curve of the pancreas' (DeFronzo 1988). *Psammomys obesus* could be divided into four separate groups based on the cross-section of glucose and insulin levels in the fed state (Figure 2). Group A (normoglycemic and normoinsulinemic), group B (normoglycemic and hyperinsulinemic), group C (hyperglycemic and hyperinsulinemic), and group D (hyperglycemic and hypoinsulinemic). In metabolic studies, we have demonstrated that group C animals have an increased body weight, increased fat deposition, increased food intake and decreased energy expenditure when compared with group A animals (Barnett et al. 1994a; 1994b; in press). As each breeding pair in our colony of *Psammomys obesus* produces an average of three to six animals per litter and these offspring can develop either group A, B, C or D characteristics, the model provides a unique opportunity to utilize new molecular biological techniques and search for novel genes linked to the development

of certain phenotypic characteristics. The new method of ddPCR described above will display all gene expression differences in tissues of diabetic versus normal animals and potentially allow the characterization of new genes associated with appearance of certain phenotypic changes such as hyperinsulinemia, increased food intake, hyperglycemia, or obesity. A recent example of the effectiveness of new molecular biological techniques in uncovering novel genes has described the positional cloning of the mouse *ob* (obese) gene and its-human homologue (Zhang et al. 1994).

It is impossible to consider genetic effects in isolation as the susceptibility loci are obviously influenced by environmental changes. The difficulty in distinguishing genetic and environmental factors is illustrated by the fact that environmental influences operating during pregnancy in utero, can mimic genetic effects (Hales 1994). In addition, a number of research groups have demonstrated the importance of nutritional factors in regulating gene expression in different animal models (Clarke and Jump 1994). Obviously, solving the genetic basis of diabetes will require further research, but results will continue to provide valuable data regarding normal cellular function as well as insights into the pathogenesis of disease. The ultimate aim of this research effort is to provide better therapies for the treatment of NIDDM.

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