Recent Progress in the Molecular Genetic Aspects of Non-Insulin-Dependent Diabetes Mellitus

Susumu Seino

Non-insulin-dependent diabetes mellitus (NIDDM) is one of the most common metabolic and endocrine disorders in developed countries. NIDDM is a multifactorial disorder in which genetic factors play a complex role in its development. Until recently, little has been known of the genetic determinants of NIDDM. Advances in molecular biological and molecular genetic approaches have now made it possible to begin to identify the diabetes susceptibility genes in NIDDM. This review describes the progress of recent research in the molecular genetic aspects of NIDDM.

Key words: diabetes mellitus, genetics, candidate genes, positional cloning, insulin

NIDDM as a genetic disease

Non-insulin-dependent diabetes mellitus (NIDDM) is among the most common of the metabolic and endocrine disorders, affecting 5–10% of the population in developed countries (1). NIDDM is characterized by impaired insulin secretion, increased basal hepatic glucose production, and peripheral insulin resistance in skeletal muscle and adipose tissue (2), but there is controversy as to which defect is primary. Some studies suggest that insulin resistance is the earliest detectable defect in the course of this disease (3–5), while others emphasize the role of abnormal β-cell function (6–9). NIDDM is a multifactorial disorder with polygenic inheritance and multiple environmental factors contributing to its development (10). Evidence of the importance of genetic factors in the development of NIDDM comes from concordance studies of NIDDM in identical (monozygotic) and nonidentical (dizygotic) twin pairs and its frequency in first-degree relatives of affected individuals (11–14). In NIDDM, the concordance in monozygotic twin pairs is less than 100%, indicating that nongenetic factors also play an important role in the etiology of NIDDM (1). Although twin, family, and population studies show that genetic susceptibility is an important determinant of NIDDM, it is evident that NIDDM is a genetically heterogeneous disorder. Several models have been proposed to explain the genetic contribution to NIDDM (15) (Fig. 1). Within individuals, the single-gene model is applied to subtypes of NIDDM such as maturity-onset diabetes of the young (MODY), which has a single autosomal dominant trait (16). Another model suggests that major genes together with several minor modifying genes represent the genetic basis for the development of NIDDM. A third model suggests that a number of different genes contribute to the development of NIDDM. Within a population, there are two models. In the first, several major genes account for a significant part of the cause of NIDDM. In the second, various different genes are responsible.

Approaches to identify the NIDDM susceptibility genes

Several approaches have been used to identify the genes of susceptibility to NIDDM.

Population-based studies and family-based studies

In population-based association studies (17, 18), the frequency of a marker, which is often a candidate gene, is compared between affected and normal subjects. If NIDDM is found to be associated with a marker more frequently than expected by chance alone, a causal relationship between the genetic variation in the marker or a locus in linkage disequilibrium with the marker and NIDDM is suggested. However, there are other explanations for positive findings (false positive results), due to population stratification and epistasis (a functional association between two loci). Also, false negative results occur when the study is insufficient to detect a difference. Despite these limitations, population association studies to identify disease-susceptibility genes are able to detect subtle genetic effects on disease susceptibility and may detect loci that are unlikely to be identified in family studies.

Two methods of family-based study are used for the identification of diabetes-susceptibility genes: linkage studies and studies of affected relative pairs (19, 20). Linkage studies are a powerful tool for identifying disease loci and have been successful in a number of single gene disorders such as cystic fibrosis (21–23) and familial polyposis coli (24, 25). In a linkage study, the cosegregation of markers and disease is tested within families; cosegregation suggests that two loci...
Within an individual

- a single gene A
- a major gene B plus a few modifying genes (a, b, ...)
- several different genes (c, d, e, ...)

Within a population

- several major genes (A, B, C, D, ...) plus different minor genes (a, b, c, d, ...)
- many different minor genes (a, b, c, d, e, ...)

**Figure 1. Possible genetic models for NIDDM.**

(one is a postulated disease-susceptibility locus, the other a marker locus at a known genomic location) are rarely separated by recombination and that they locate close together in the genome. However, linkage studies cannot as readily be applied in the analysis of the genetics of complex disorders, such as the common forms of NIDDM, as they are in a simple monogenic diabetes such as MODY, which shows early onset and autosomal dominant inheritance (16). However, it is also possible to simplify studies of the genetics of complex disorders by analysis of only affected members of families. One, which has been widely used, is analysis of affected sib-pairs (19, 20). This involves studying affected sibs in a pedigree to see how frequently a particular copy of a chromosome region is shared identical-by-descent (IBD), that is, inherited from a common ancestor within the pedigree. This method requires no information of mode of inheritance, allele frequencies, or penetrance in advance. The affected sib-pair analysis has been used successfully in complex diseases such as hypertension and insulin-dependent diabetes mellitus (IDDM) (26). However, at least 300 affected relative pairs are thought to be required to find loci linked to disease.

*The candidate gene approach and the positional cloning approach*

One approach to identify a gene causing NIDDM is the “candidate gene” approach (27). This method examines the loci encoding proteins that are involved in the regulation of blood glucose levels, including insulin secretion and insulin action, and requires a clear understanding of the molecular basis of insulin secretion and its action. The current models of the mechanisms of insulin secretion and insulin action are illustrated in Figs. 2 and 3. Insulin secretion is regulated by many factors, including fuels, hormones, and neurotransmitters (28–30). Among these regulatory substances, glucose is the most important. The model in which the stimulatory effect of glucose on insulin secretion depends on the rate of glucose metabolism, called the “glucose metabolism hypothesis” (31, 32), has generally been accepted. Glucose is first transported into pancreatic β-cells through the glucose transporter GLUT2 and subsequently phosphorylated by glucokinase, the rate-limiting step in glucose metabolism (33). Increased glucose metabolism leads to an increase in adenosine triphosphate (ATP). An increase in ATP inhibits the ATP-sensitive K⁺-channels, which link metabolic status to the membrane potential of pancreatic β-cells (34). As a result, the β-cell membrane is depolarized, leading to the opening of voltage-dependent calcium channels, allowing calcium influx into the β-cells. A rise in the intracellular calcium concentration triggers insulin secretion (28). Accordingly, the genes whose products are expressed specifically in pancreatic β-cells and are involved in glucose-induced insulin secretion are good candidates for NIDDM susceptibility genes.

Insulin exerts multiple effects on the cells of target tissues. Although many signaling pathways are involved in the insulin actions, understanding the molecular mechanisms by which insulin stimulates glucose transport in muscle and adipose
tissue is most important for selecting candidate genes for susceptibility to NIDDM (9). Insulin receptors play a central role in insulin actions (5, 35, 36). The insulin receptor is a transmembrane protein with intrinsic tyrosine kinase activity. The binding of insulin to its receptor causes insulin receptor autophosphorylation (5, 36) and phosphorylation of cellular substrates such as IRS-1 (5, 36). IRS-1 binds to several proteins containing src homology 2 (SH2) domains, one of which is the p85 subunit of phosphatidylinositol (PI)-3-kinase (37). PI-3 kinase, a heterodimeric protein is comprised of a catalytic p110 subunit and a regulatory p85 subunit is a likely candidate in the signaling pathway of translocation of the glucose transporter isoform GLUT4, which is responsible for insulin-stimulated glucose transport in muscle and adipose tissue (38, 39). Accordingly, the genes whose products are involved in insulin actions, especially involved in signaling pathways in GLUT4 translocation, make good candidates for NIDDM susceptibility genes.

Another approach currently used to identify NIDDM susceptibility genes is “positional cloning” (27). When candidate genes have not been localized on the genome or it is not known which gene may be involved in a disease, the positional cloning (also referred to as “reverse genetics”) approach can be used to
identify NIDDM susceptibility genes in families. A linkage analysis of multiple affected families is usually the first step: families segregated for disease are typed at a large number of highly polymorphic DNA markers throughout the genome and regions linked to the disease are identified. Once such regions are identified, special molecular biological techniques such as the isolation of DNA from a cosmid or YAC (yeast artificial chromosomes) library are employed to identify the NIDDM locus itself. This method has successfully been used for gene identification in simple Mendelian disorders (27). Application of this method to MODY, although the genes exactly at the loci have not yet been determined, has recently identified a region on chromosome 20q (MODY1 gene) (40) and a region on chromosome 12q (MODY3 gene) linked to glucose intolerance (41).

Genomic information obtained from rodent models may also be applied to the human genome: human chromosomal regions syntenic with regions thought to be NIDDM susceptibility genes in rodent models of NIDDM would be good candidates. A good example is the obese gene (ob gene) recently cloned from the ob/ob mouse (42). The ob gene was cloned by the positional cloning method and, subsequently, its human homolog also was isolated by homology screening. The genetic information obtained by “mRNA differential display” (43) may also be helpful in selecting candidates for human NIDDM susceptibility genes.

The NIDDM susceptibility genes

Despite the extensive effort using all these methods, only a small fraction of the NIDDM susceptibility genes has been identified to date.

Insulin gene

The human insulin gene is located near the short arm end of chromosome 11 in band p15.5 and has 3 exons (44, 45). Although defects in the insulin/proinsulin molecule could influence insulin biosynthesis, processing, and regulated secretion as well as the biological potency of insulin, almost all NIDDM patients have structurally normal insulin/proinsulin. Mutations in the insulin gene so far have been reported in less than ten families (46, 47). Thus, an insulin/proinsulin mutation would seem to be a rare cause of NIDDM in most populations. The mutant insulins bind to the insulin receptor with decreased affinity. The only exception is Asp B10 insulin. Although the Asp B10 proinsulin detected in a patient with hyperproinsulinemia (proinsulin Providence) was shown to be processed into normal Asp B10 insulin, which is rapidly degraded within the β-cells, synthesized human insulin with Asp B10 insulin has been shown to have the 4 to 5 times normal binding affinity and biological potency (48). Mutations in the promoter region of the insulin gene could also affect the regulation of the insulin gene and lead to a decrease in transcription rate. Recently, a new variant allele of the promoter region in the insulin gene has been identified in about 5% of African American NIDDM patients (49). This variant is shown to cause a significant decrease of transcription activity, suggesting that a mutant insulin promoter allele might contribute to impaired insulin synthesis, at least in this subgroup of the NIDDM population.

Insulin receptor gene

Insulin resistance is one of the major phenotypic characteristics of NIDDM, at least in patients in the USA (3–5). It has long been suggested that syndromes of extreme insulin resistance such as leprechaunism, Rabson-Mendenhall syndrome, and type A severe insulin resistance are associated with the expression of abnormal insulin receptor molecules (50). Since the cloning of the insulin receptor cDNA (51, 52) and, subsequently, the insulin receptor gene (53), it has become possible to confirm that mutations in the insulin receptor gene are the causes in these patients. The human insulin receptor gene spans more than 120 kilobase pairs (kbp) on the short arm of chromosome 19 in the region of bands p13.3 – >p13.2 (54) and is comprised of 22 exons (53). Mutations of the insulin receptor gene were first identified in a patient with Rabson-Mendenhall syndrome (55) and in a patient with leprechaunism (56). Using polymerase chain reaction (PCR)-based strategy (57), more than 50 different mutations of the insulin receptor gene have so far been identified (58). These mutations are associated with either a dominant or a recessive autosomal mode of transmission of insulin resistance, depending on the nature of the insulin receptor. The mutations have been divided into five classes on
the basis of the mechanism by which they impair insulin receptor function (58): 1) impaired receptor biosynthesis, 2) impaired transport of receptors to the cell surface, 3) decreased affinity of insulin binding, 4) impaired tyrosine kinase activity, and 5) accelerated receptor degradation. Patients with insulin resistance due to mutations in the extracellular domain of the insulin receptor are genetic compounds or homozygous for the same mutation. By contrast, most of the patients with insulin resistance due to a mutation in the tyrosine kinase domain also express normal insulin receptors, suggesting that these mutations appear to act as a dominant negative mutation. The contribution of mutations in the insulin receptor gene to the common forms of NIDDM is unknown. Taylor et al have suggested that the prevalence of mutations in the insulin receptor gene is at least 0.1% of the general population and, therefore, 1 to 10% of patients with NIDDM may have mutations at the insulin receptor locus (58), but the prevalence of mutations in the insulin receptor gene in the common forms of NIDDM is not known.

**MODY genes**

(a) **MODY1 gene.** Fajans has studied the RW pedigree, an American family of German origin since 1958 (16). The segregation of NIDDM in this family is consistent with autosomal dominant inheritance. Bell et al tested seventy-six loci for linkage with MODY in the RW pedigree and found that the adenosine deaminase gene (ADA) on the long arm of chromosome 20 cosegregates with MODY in this family (40). The gene responsible for the MODY in the RW pedigree is now called the “MODY1” gene. Most subjects with NIDDM in the RW family who have inherited the risk allele of the MODY1 gene appear to have a characteristic pattern of reduced and delayed insulin secretory response to glucose, suggesting that the MODY1 gene product may be involved in glucose-induced insulin secretion. The MODY1 gene has not yet been identified, but efforts are being made toward isolating the MODY1 gene by positional cloning.

(b) **MODY 2 gene (glucokinase gene).** Glucokinase (ATP: D-glucose-6-phosphotransferase), expressed exclusively in pancreatic islets and liver, catalyzes the first step of glycolysis, regulating the insulin secretion in response to glucose in pancreatic β-cells and the uptake of glucose in the liver (33). In contrast to other hexokinases, it has a high Km value and is not inhibited by glucose-6-phosphate. Rates of glucose phosphorylation vary with glucose levels, suggesting that glucokinase acts as a “glucose sensor” (33). The human glucokinase gene is composed of 12 exons spanning a region of more than 50 kbp of chromosome band 7p13 (59–61). It has two tissue-specific promoters, one of which is active in pancreatic β-cells and the other in liver (62–64). Linkage between the glucokinase gene and diabetes was first reported in French families with MODY (65), and, subsequently, mutations of the glucokinase gene have been shown to be the cause of MODY (66). Almost 30 different mutations have been identified in the glucokinase gene in diabetes mellitus (67, 68). The majority of the mutations have been found in MODY. They also have been found in gestational diabetes mellitus (69, 70) and in late-onset NIDDM (71–73). Glucokinase mutations have been identified in exons 2-10, with the majority being clustered in exons 5, 7, and 8. All of the glucokinase mutations associated with MODY result in reduced enzymatic activity with a decreased Vmax and/or increase Km for glucose (74). Since glucokinase is a monomer, it seems unlikely that the mutant glucokinase acts as a dominant negative mutation. Rather, a gene-dosage mechanism is the most likely explanation for a dominantly inherited form of diabetes caused by glucokinase mutation. It has been estimated that a 15% decrease in glucokinase activity could shift the threshold for glucose-stimulated insulin secretion form 5 to 6 mM glucose (33). Although glucokinase mutations are a major cause of MODY, at least in the French families, screening for mutations in NIDDM patients indicates that the glucokinase gene is unlikely a major susceptibility gene for the common late-onset NIDDM. The glucokinase gene is now referred to as the “MODY2” gene.

(c) **MODY3 gene.** It has been suggested recently that there is a subset of French MODY families in which the diabetes is not linked to the ADA locus (MODY1) or to the glucokinase gene (MODY2), rather, it is linked to microsatellite markers on chromosome 12q (41). These MODY patients exhibit hyperglycemia with a severe impairment of insulin secretion, suggesting that the gene responsible for MODY3 is involved in insulin secretion (41).

**Mitochondrial DNA**

Several syndromes caused by point mutations, deletions, or duplications of mitochondrial DNA (mt DNA) are characterized by a decrease of oxidative phosphorylation and are associated with diabetes (75, 76). It has recently been reported that a single mutation (G for A at nucleotide 3243, A3243 mutation) in the mt DNA for the tRNALeu is cosegregated with diabetes and deafness in patients having maternally inherited diabetes (77, 78). The cause of the diabetes is thought to be probably a chronic failure of oxidative phosphorylation in the pancreatic β-cells, leading to impaired insulin secretion. Screening of mt DNA mutations in Japanese NIDDM patients has suggested that approximately 1% of NIDDM in Japan may be associated with the A3243 mutation (79). The same mutation is also found in patients with MELAS (mitochondrial myeloencephalopathy lactic acidosis stroke-like episodes) syndrome, a neuromuscular disease accompanied by deafness and diabetes. Although the reason for the differences in phenotypic expression between MELAS and diabetes with deafness is unknown, it might be due to the varying degree of heteroplasmy in the different tissues.

**Other candidates for NIDDM susceptibility genes**

(a) **The susceptibility genes of β-cell dysfunction.** The glucose transporter GLUT2, having a high Km for glucose, is expressed predominantly in pancreatic β-cells and liver, and is thought to be involved, in part, with glucose-sensing in insulin secretion (80–82). Defects in the GLUT2
gene, therefore, could lead to glucose-sensing abnormalities in pancreatic β-cells and/or abnormal glucose release from the liver. However, in genetic studies of GLUT2 no significant mutations in most patients with NIDDM have been found (83, 84), suggesting that the GLUT2 gene is not a susceptibility gene for the common forms of NIDDM.

The voltage-dependent calcium channels in pancreatic β-cells are the major pathway of the increase in intracellular calcium in response to glucose, the primary signal for insulin secretion. Therefore, they are a candidate gene for susceptibility to NIDDM (85, 86). Recently, we have identified the β-cell ATP-sensitive K⁺ (K$_{ATP}$) channel (87), a key molecule linking glucose metabolism to the membrane potential of β-cells. The β-cell K$_{ATP}$ channel is a complex of two subunit proteins encoded by the inward rectifier subunit BIR gene and the sulfonylurea receptor gene (87). Whether or not the β-cell K$_{ATP}$ channel genes are NIDDM susceptibility genes remains to be determined.

Amylin (islet amyloid peptide) is a 37 amino acid peptide (88, 89) and is shown to be cosecreted with insulin from pancreatic β-cells (90). Since amylin is the major protein component of islet amyloid seen in pancreatic islets of NIDDM patients, the deposition of which may contribute to the impaired β-cell function in NIDDM, a primary defect of the amylin gene has been postulated. However, several studies have failed to detect any mutations in NIDDM patients, indicating that a primary structural abnormality of amylin or its precursor is not responsible for the formation of the islet amyloid seen in NIDDM patients (91, 92). Other candidates are the genes for the receptors for hormones called “glucoincretins” such as GLP-1 (93) and GIP (94), since these hormones have strong potentiating effects on glucose-induced insulin secretion (95). Defects in these receptors (96) at the level of pancreatic β-cells might lead to impaired glucose-induced insulin secretion.

(b) The susceptibility genes of insulin resistance

The insulin-responsive glucose transporter GLUT4 is responsible for insulin-stimulated glucose uptake into fat and muscle (35, 96). Defects in GLUT4 activity or expression could account for the defective glucose transport into these tissues which is seen in NIDDM. However, a genetic study of GLUT4 failed to reveal significant mutations in most patients with NIDDM (97), suggesting that a genetic abnormality of GLUT4 is unlikely the cause of the common forms of NIDDM.

Glycogen synthase is one of the target enzymes of insulin and its activity has been found to be reduced in patients with NIDDM (98, 99). Although restriction fragment length polymorphisms (RFLP) generated by Xba1 digestion have been shown to be associated with diabetes in Finns (100), recent studies show no significant mutations in the glycogen synthase gene of NIDDM patients (101, 102). The reduction of glycogen synthase activity found in NIDDM, therefore, may be secondary to the reduced activation of glycogen synthase phosphatase.

The gene encoding the first substrate for the insulin receptor kinase IRS-1 (5, 36) has been considered a candidate. Several amino acid sequence variations in IRS-1 have been identified (103–106), but the frequencies of the variations in NIDDM and in normal subjects were not statistically different in the different populations.

Recently, the obese gene (ob gene) has been cloned (42). The expression of the obese gene product, called “leptin”, is thought to be a signaling factor regulating body weight homeostasis and energy balance (107–109). Since obesity is an important factor that predisposes to NIDDM, it will be interesting to learn how the ob gene contributes to the development of NIDDM with obesity. A genetic variation of the β3-adrenergic receptor (replacement of tryptophan by arginine at position 64) is also thought to be associated with obesity (110–112).

Concluding remarks

NIDDM is not a single disease, but a clinical syndrome characterized by chronic hyperglycemia and a predisposition to develop chronic complications including retinopathy, nephropathy, and macrovascular disease. It is clear that genetic factors play a major part in the development of NIDDM, but NIDDM appears to be a polygenic disease with a complex pattern of inheritance. The common forms of NIDDM are likely to be heterogeneous conditions in which the specific genetic determinants differ between individuals and among different races. This genetic complexity justifies diabetes being referred to as “the geneticist’s nightmare”. Only recently have advances in molecular biology and molecular genetics made it possible to begin to clarify the genetic basis of NIDDM. Molecular biology also has provided a better understanding of the molecular mechanisms of insulin secretion and its action, thereby facilitating genetic studies of NIDDM by the candidate gene (27) and positional candidate gene approaches (113). Whether there are major diabetes genes responsible for the development of NIDDM or whether NIDDM is a multigenic disease remains to be resolved.

Acknowledgements: Our studies were supported by Scientific Research Grants from the Ministry of Education, Science and Culture and from the Ministry of Health and Welfare, Japan; a grant from the Yamanouchi Foundation for Research on Metabolic Disorders. I thank M. Tanemura for preparing the manuscript.

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Molecular Genetic of NIDDM


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Molecular Genetic of NIDDM

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