Candidate Genes for Insulin Resistance

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Insulin resistance confers increased susceptibility to NIDDM, atherosclerotic cardiovascular disease, ovarian hyperandrogenism, and possibly hypertension. Insulin resistance is largely inherited, in rare cases as a monogenic disorder or more commonly as a complex trait. The search for insulin resistance genes relies mainly on two complementary approaches: *1*) positional cloning using random DNA markers present throughout the genome; and 2) the analysis of specific candidate genes. This report briefly summarizes the candidate gene approach to insulin resistance. Progress related to the analysis of genes encoding molecules that participate in insulin action is reviewed. In addition, the spectrum of potential genetic defects that might contribute to insulin resistance, both at the level of the target cell and secondarily (e.g., obesity genes), is discussed.

Insulin resistance is an extremely common pathophysiological trait that is implicated in the development of NIDDM, atherosclerotic cardiovascular disease, hypertension, dyslipidemias, and the polycystic ovarian syndrome (1,2). Several lines of evidence clearly point to genetic factors as important determinants of insulin sensitivity. These findings can be summarized as follows. *1*) The prevalence of (insulin-resistant) NIDDM varies dramatically among different racial and ethnic groups; it is markedly higher in certain native American (e.g., Pima Indians) or South Pacific (e.g., Nauruans) populations in which the foreign genetic admixture results in lower risk (3–5). *2*) The degree of in vivo insulin sensitivity varies widely between lean healthy subjects, matched for age and physical activity (6). *3*) Insulin resistance is a defect that appears very early in the clinical course of NIDDM (7); moreover, NIDDM per se has a strong genetic basis (4,8). *4*) Insulin resistance is an independent familial trait that predicts the development of NIDDM (4,9–12). *5*) Several rare syndromes of extreme insulin resistance are clearly inherited as either dominant or recessive single-gene disorders (13).

Although increased nutrient intake and subsequent obesity is a prominent cause of insulin resistance, there is emerging evidence suggesting that obesity per se (or even altered body fat distribution) is, at least in part, genetically determined (14). Importantly, clinically significant insulin resistance may often occur when individuals with what was once a favored thrifty genotype (reduced satiety, more efficient fuel storage) are exposed to a sedentary lifestyle accompanied by increased food availability and advancing age (5).

POSITIONAL CLONING VERSUS ANALYSIS OF CANDIDATE GENES — The search for specific genetic defects that contribute to reduced insulin sensitivity and confer susceptibility to NIDDM is complicated by the fact that this heterogeneous syndrome is usually polygenic and may be clinically apparent only when present in combination with appropriate environmental factors (3,8,15). Furthermore, physiological parameters including glycemia, insulinemia, or more sophisticated indexes of insulin sensitivity are continuous variables within the population, so it may be difficult to distinguish affected from unaffected subjects (15). Segregation analysis has been used to try to determine the number and mode of inheritance of genes that affect insulin sensitivity (16). However, such methods can be easily biased by selection criteria and may not accurately reflect the true number of genetic loci that contribute to a complex trait (17).

Positional cloning is a powerful strategy for identifying disease-causing genetic defects. This involves using linkage analysis techniques to assess coinheritance of the disease with DNA markers spaced evenly across the genome (15,17). These methods have been tremendously successful when applied to classical monogenic disorders (e.g., cystic fibrosis), in which disease genes can be cloned eventually based only on their chromosomal location (17). Since classical linkage analysis requires accurate estimates of parameters such as penetrance and mode of inheritance (17), its application is therefore limited in the case of common forms of insulin resistance. A combination of the following strategies will facilitate the total genome approach to detection of new NIDDM and/or insulin resistance genes: *1*) refined techniques that are better suited to the study of complex traits and *2*) greater emphasis on the study of selected populations (e.g., Pima Indians) and discrete subphenotypes (e.g., impaired nonoxidative peripheral glucose disposal [see below]) (8,15,17). Affected sib-pair analysis is an approach that has traditionally been used to identify genetic loci that underlie a dichotomous trait for which individuals are classified either as affected or unaffected (17). However, this method can be applied to quantitative traits and may be improved by selecting discordant sib-pairs: those who lie near opposite ends of the spectrum of insulin sensitivity (17,18). An exciting new development involves the use of sib-pair linkage analysis to identify two genetic loci (chromosomes 4q and 7q), which appear to confer impaired insulin-mediated in vivo glucose uptake (i.e., insulin resistance per se) in Pima Indians (19,20).

An alternative strategy is the focused study of specific candidate genes. Thus, a rapidly expanding list of candidate genes for insulin resistance encode molecules that participate in the in vivo

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FFA, free fatty acid; SSCP, single-strand conformation polymorphism; TNF-α, tumor necrosis factor-α.

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Table 1—*Analysis of candidate genes for insulin resistance*

Gene	Methods/results	References
Insulin receptor	Cloning and sequencing, SSCP scanning and sequencing, expression of mutant receptors in cells and transgenic mice; >35 different mutations found in patients with severe insulin resistance; functional significance of mutations well established. Rare in typical NIDDM or moderate insulin resistance	25
Insulin receptor substrate 1 (IRS-1)	SSCP scanning and analysis of variant frequency; five infrequent amino acid changes, Gly^{972}→Arg possibly ↑ in NIDDM	34,35
Ras associated with diabetes (RAD gene)	mRNA subtraction analysis and population association; possible ↑ mRNA in skeletal muscle and possible association of polymorphisms with NIDDM	36
Insulin-stimulated protein kinase 1 (ISPK-1, *rsk*2)	SSCP scanning; one uncommon amino acid variant not associated with insulin resistance; normal muscle mRNA expression in NIDDM	37
Protein phosphatase 1		
G-subunit	SSCP scanning; two amino acid variants: the common Asp^{905}→Tyr may be associated with ↓ nonoxidative glucose disposal; normal muscle mRNA in NIDDM	28,38
Catalytic subunits α, β, γ	SSCP scanning; no amino acid variants and normal muscle mRNA expression in NIDDM	37
Glycogen synthase	SSCP scanning and population association studies; one rare amino acid mutation; one common polymorphic marker associated with NIDDM; possible ↓ in muscle mRNA in NIDDM; several promoter variants exist but no relation to ↓ mRNA expression	4,27,39
Glucose transporters		
GLUT1	Population association and linkage studies with NIDDM pedigrees; largely negative findings	40
GLUT4	SSCP scanning and several population association studies; two amino acid variants with unknown functional impact; three promoter variants with no clear functional impact; normal muscle mRNA levels in NIDDM	27,41,42
Hexokinase II (HKII)	SSCP scanning and analysis of variant frequency; one common and three rare amino acid variants; no clear association with insulin resistance or NIDDM	43,44
Fatty acid–binding protein 2 (FABP2)	Linkage analysis and association studies; positive linkage with insulin resistance in Pima Indians; weak or no association in white NIDDM patients	19,24,45

Several potential candidate genes for insulin resistance have been evaluated. Experimental approaches have included cDNA cloning and sequencing, molecular scanning using SSCP, population association studies, linkage analysis with affected pedigrees, and measurement of gene expression in relevant tissues.

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actions of insulin (Fig. 1) or may secondarily be involved in the regulation of insulin sensitivity. Having a newly cloned candidate gene in hand allows the investigator to use one or more approaches to test for the presence of potential disease-causing mutations. *1*) Polymorphic markers in or near the gene can be used to study affected pedigrees (linkage analysis or sib-pair analysis) or in population association studies to test for coinheritance or association of gene variants with the disease. *2*) With the polymerase chain reaction, relevant portions of the gene can be isolated from affected subjects and directly sequenced or screened for nucleotide variation using a molecular scanning approach (21). Scanning techniques such as single-strand conformation polymorphism (SSCP) allow for rapid determination of nucleotide variation with ≥90% sensitivity (22,23).

Although numerous potential mutations in candidate genes may be detected (Table 1), proving the clinical significance of a given putative mutation relies on one or more of the following criteria: *1*) functional defects that are evident after expression of the mutant gene using an in vitro system (or transgenic animals); *2*) cosegregation of the mutation with a relevant phenotype in affected pedigree(s); and *3*) increased frequency of the mutation in affected subjects versus well-matched normal control subjects. The distinction between silent changes and pathogenic mutations is especially difficult, since defects involving more than one gene are likely to be necessary at least in certain cases.

It is important to note that positional cloning and candidate gene approaches are truly complementary. Thus, identification of a candidate chromosomal locus using a total genome search is most often followed by an assessment of relevant candidate genes, which have been previously mapped to the vicinity of the locus in question (17). An illustration of this point involves identification of a point mutation in the intestinal fatty acid–binding protein gene (located on chromosome 4q), which was discovered after linkage between markers on 4q and insulin resistance in Pima Indians was found (see above) (Table 1) (19,24).

SPECTRUM OF POTENTIAL CANDIDATE GENES FOR INSULIN RESISTANCE — As depicted in Fig. 1, insulin action is a complex process that involves numerous molecules including those that mediate insulin's cellular signals and proteins that catalyze glucose uptake and metabolism. In addition to potential target cell defects,

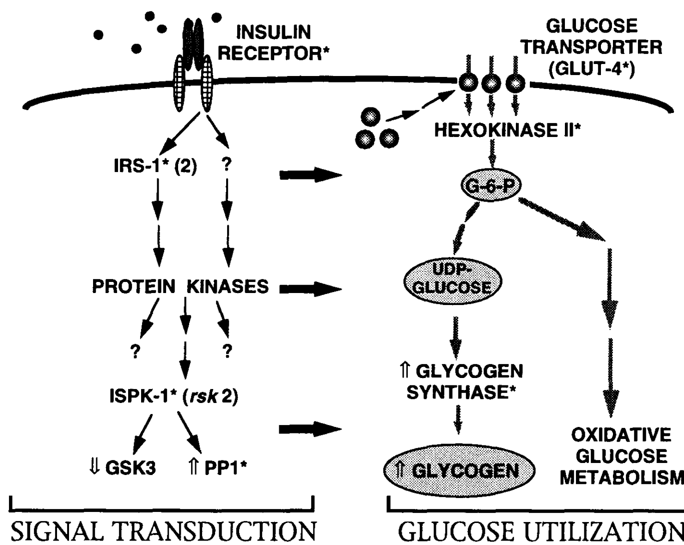

Figure 1—*Several key steps in peripheral insulin action: potential genetic loci for insulin resistance at the level of the target cell. Peripheral insulin-stimulated glucose disposal (e.g., in a muscle cell) involves molecules that mediate insulin signaling and proteins that catalyze glucose uptake/metabolism. GLUT4 is the major glucose transporter isoform expressed in insulin-responsive peripheral tissues. In response to unknown components of insulin signaling, GLUT4 translocates from an intracellular pool to the cell surface where it facilitates glucose entry. Glucose metabolism involves numerous enzymatic steps including conversion of glucose to glucose-6-phosphate (G-6-P) by hexokinase II (the major isozyme in muscle and fat), conversion of UDP-glucose into glycogen by glycogen synthase (nonoxidative glucose metabolism), and multiple steps in glucose oxidation. Signaling molecules include the insulin receptor itself and insulin receptor substrate 1 (IRS-1) (a related molecule known as IRS-2 also exists). A specific protein kinase known as ISPK-1 or* rsk 2 *may modulate the functions of glycogen synthase kinase 3 (GSK3) and protein phosphatase 1 (PP1), which then promote glycogen synthase activation. Multiple additional protein kinases are also activated as well as other classes of signaling molecules (such as phosphatidylinositol-3-kinase). Gene products noted by asterisks have been analyzed in subjects with NIDDM or insulin resistance (Table 1).*

a growing number of additional gene products are implicated as secondary factors that could influence insulin sensitivity. Thus, there is a broad array of genes that might harbor mutations conferring impaired insulin sensitivity.

Insulin receptor mutations

Studies of cells derived from patients with several syndromes of extreme insulin resistance led to the discovery of functional defects involving the insulin receptor (13). Subsequently, a number of specific mutations in the insulin receptor gene were identified. To date, more than 35 different mutations have been described, mostly affecting subjects with one of three unusual syndromes: type A syndrome, Rabson Mendenhall syndrome, and leprechaunism (13,25). Although receptor mutations probably account for all cases of leprechaunism (and the Rabson Mendenhall syndrome), only a subset of insulin-resistant subjects with features of type A syndrome have mutations in this gene; furthermore, receptor mutations are rarely present in patients with common NIDDM (22,25,26). Nevertheless, insulin receptor mutations represent the only clearly defined genetic cause of insulin resistance in humans.

Additional intrinsic target cell defects

Recent advances in our understanding of insulin action have led to the cloning of several genes that encode potential insulin-signaling intermediates as well as enzymes involved in peripheral glucose metabolism (Fig. 1). The existence of genetic defects involving these or related molecules is nearly certain, since patients with inherited defects in target cell insulin responsiveness (without insulin receptor mutations) have been described (13,26).

Current knowledge of the pathophysiology of common forms of insulin resistance suggests the existence of defects involving either *1*) glucose transport and/or phosphorylation or 2) nonoxidative glucose storage as glycogen (4). Thus, recent attention has been focused on the genes encoding molecules such as GLUT4 glucose transporters, hexokinase II, and glycogen synthase (Fig. 1 and Table 1). In addition, genes for specific signaling molecules such as ISPK-1 and PP1 that are implicated in the regulation of glycogen synthase have been scrutinized. Despite intensive efforts, the results of a number of studies have been relatively disappointing (Table 1). Thus, no additional gene (or genes) has emerged as a major locus responsible for inherited insulin resistance. The studies cited in Table 1 suffer from several limitations: *1*) in most cases, only a limited number of subjects were included; 2) with some exceptions (27), the possibility of mutations affecting gene promoter regions has not been addressed; and 3) potential mutations may have been missed because of imperfect molecular scanning sensitivity. The finding of a positive association between a mutation in the PP1 (G-subunit) gene and reduced nonoxidative glucose disposal is a hopeful sign (28) (Table 1). This study suggested that an interplay between more than one genetic variant might often be required for clinically significant insulin resistance. Moreover, the study provides a paradigm for the focused use of genetic screening in subjects characterized by more detailed phenotype analysis.

Other factors (extrinsic to the target cell)

Our knowledge of secondary physiological parameters that modulate in vivo insulin sensitivity has expanded to the point at which one can now envision a potential pathogenic role for specific gene products. For example, adipocyte-derived tumor necrosis factor-α (TNF-α) (29) or free fatty acids (FFAs) (30) have both been implicated in mediating the insulin resistance of obesity. Could genetic defect(s) result in a primary increase in circulating TNF-α or FFAs? Indeed, the rare autosomal recessive disorder known as congenital generalized lipoatrophy (lipoatrophic diabetes) is characterized by insulin resistance and markedly high levels of triglycerides and FFAs (13). Since it remains unclear whether the syndrome results from a failure to store fat or from accelerated lipolysis, plausible candidate genes may include factors that mediate fat cell differentiation and enzymes that regulate lipogenesis or lipolysis. Interestingly, genetic variation at or near the lipoprotein lipase gene locus was found to

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be associated with features of the insulin resistance syndrome (elevated insulin and triglyceride levels with reduced HDL cholesterol), although mutations in this gene have yet to be described as a cause of insulin resistance (31). An alternative inherited basis for insulin resistance might result in impairment of insulin's ability to modulate the nitric oxide synthase system, since this seems to contribute to insulin-stimulated glucose disposal by inducing vasodilatation of muscle vasculature (32).

It is obvious that any genetic defect leading to the development of obesity would naturally qualify as a prominent insulin resistance gene as well. This is illustrated by the discovery that mutation of the newly cloned *ob* gene, which encodes a putative satiety factor, causes marked obesity and severe insulin resistance in affected mice (33). Although specific genetic causes of human obesity have yet to be described, mutations in the human *ob* counterpart or genes encoding related molecules are obvious candidates that are worthy of study.

CONCLUSIONS— Rapid progress in elucidating the molecular mechanism of insulin's actions on key target tissues such as muscle and in defining molecular mechanisms for insulin resistance is now yielding an expanding spectrum of potential insulin resistance genes. Analysis of such candidate genes will gradually enable us to define the inherited causes of insulin resistance when combined with *1*) a total genome search for new chromosomal loci that contribute to this complex trait, and *2*) the focused study of subjects with discrete biochemically characterized physiological defects.

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