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PROSPECT

Pima Indians as a Model to Study the Genetics of NIDDM

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Abstract More than half the Pima Indians over 35 years of age have non-insulin dependent diabetes mellitus (NIDDM). They have been the focus of prospective epidemiologic and metabolic studies for over two decades and the data collected during these studies are now proving invaluable in efforts to find genetic markers for NIDDM in humans. The Pima Indian model of this disease affords two major advantages. The population is genetically homogeneous compared to Caucasian populations, and therefore the causes of NIDDM are less heterogeneous, simplifying genetic linkage studies. Equally important, based on results from metabolic studies, two pre-diabetic phenotypes have been identified in the Pimas: insulin resistance and a low metabolic rate. Use of these phenotypes in genetic linkage analyses should greatly improve chances of finding genetic markers for NIDDM since these phenotypes may be more closely related to the putative abnormal gene products, and actual disease genes, than is the hyperglycemia of the fully developed phenotype of NIDDM.

Key words: diabetes mellitus, genetics, Pima Indians, insulin resistance

There are currently about ten million persons with diabetes mellitus in the United States [11]. About 80% of these people have non-insulin dependent diabetes (NIDDM), previously called maturity-onset diabetes [11]. Several lines of evidence have established NIDDM as a disease with significant genetic determinants. The disease shows strong familial aggregation and its prevalence varies among different ethnic groups living in the same environment [15,33]. The most convincing proof of major genetic determinants of NIDDM is the high concordance rate of the disease among monozygotic twins (60–90%) as compared to dizygotic twins [1,22].

NIDDM is much more prevalent among Native Americans than among American Caucasians, African-Americans, or Hispanic-Americans. In particular, the Pima Indians of Arizona have the highest reported prevalence of the disease of any population in the world [15]. More than half of the Pimas over the age of 35 years have the disease [15].

Because of the extraordinarily high prevalence of NIDDM and its complications, the Pimas have been the focus of epidemiologic and metabolic investigations since 1965. Since that time, considerable information has been gathered regarding the etiology and pathogenesis of NIDDM. This large and growing data base is now proving invaluable in efforts to identify the genetic determinants of NIDDM in humans. In this brief report, we discuss recent problems and progress in attempts to find a genetic marker(s) for NIDDM in the Pima Indians.

PROBLEMS OF PHENOTYPE

Among the Pimas, and as subsequently shown in other populations, the plasma glucose concentration 2 hours after ingesting 75 gms of glucose has a bimodal frequency distribution [15]. The intersection of the two distributions occurs at a plasma glucose concentration of ~200 mg/100 ml and above this glucose concentration the prevalence of diabetic complications increases [15]. For these reasons, the criterion for the diagnosis of diabetes mellitus was established as a 2-hour post-load glucose concentration of 200

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mg/100 ml [8]. The diagnosis of type II diabetes mellitus, or NIDDM, as distinct from type 1 diabetes, or insulin-dependent diabetes, is then made by establishing the lack of an absolute requirement for insulin therapy.

The phenotype of NIDDM, defined in this way, has been used in genetic association and linkage studies to find genetic markers for the disease. However, despite reports of weak associations [6,14,16,20,26,29,32,34], a clear genetic marker for the disease has not yet been found.

One possible explanation for these negative results is that within a Caucasian population, derived from very diverse genetic origins, a hyperglycemic response to oral glucose results from a heterogeneous group of metabolic abnormalities. If this metabolic heterogeneity results from genetic heterogeneity, a search for a common gene will be difficult, if not impossible. In this regard, a major advantage of studying the Pimas is that they have been relatively geographically isolated for hundreds of years and are more genetically homogeneous than Caucasians. Therefore, it is likely that the same metabolic abnormality, or the same constellation of specific metabolic defects, results in NIDDM in all Pimas. This greatly increases the chances of successfully identifying a genetic marker, or markers, for the disease.

Another possible explanation for the previously unsuccessful attempts to identify a genetic marker for NIDDM is that the phenotype, hyperglycemia, is metabolically too "distant" from the underlying genotype(s). A classic example of this potential problem is illustrated by Penrose's data of the frequency distribution of several phenotypic characteristics of individuals with and without phenylketonuria [24 as also reviewed in 7]. Classic phenylketonuria is a disease that results from genetic mutations in the gene for phenylalanine hydroxylase which converts phenylalanine to tyrosine. The resulting excess blood levels of phenylalanine clearly distinguish affected from unaffected individuals. The disease also causes several other characteristic abnormalties. As shown in Figure 1, however, as the phenotypic characteristics used to define the disease become further removed from the abnormal gene product it becomes increasingly difficult to distinguish affected from non-affected individuals. For example, using head size or hair color as the disease phenotype, rather than plasma phenylalanine levels, does not permit a precise definition of affected individuals.

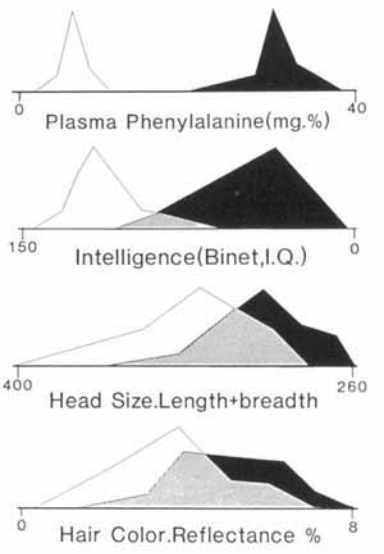

Fig. 1. Frequency distributions in control populations (unshaded area) and in phenylketonurics (black area). The hatched area represents area of overlap between the two distributions. Notice that if the disease called *phenylketonuria* is defined on the basis of the plasma phenylalanine concentration, affected and non-affected individuals can be precisely identified. However, if phenotypic characteristics such as intelligence quotient, head size, or hair color are used to define the disease it is much less clear who is affected or not because the phenotypic characteristics (intelligence quotient, head size, and hair color) are more metabolically distant from the pathogenic genotype. The expression of these more distant phenotypic characteristic is influenced by other genetic factors as well as by environmental factors. The best chance of finding a genetic marker for phenylketonuria was to use a phenotypic characteristic that most clearly distinguished affected and non-affected individuals—that is, plasma phenylalanine concentrations. Hyperglycemia is a phenotypic characteristic of persons with NIDDM, much like hair color in phenylketonurics, that is metabolically distant from the pathogenic genotype we are trying to identify. Chances of finding a genetic marker for NIDDM would be greater if there was a phenotypic characteristic that more clearly distinguished affected from non-affected persons. Redrawn with permission of Cambridge University Press [24].

This is due to the combined effect of other genetic and environmental factors to influence the more distant phenotypic characteristics such as hair color. If attempts had been made to find a single genetic marker for this disease, using phenotypic characteristics to define the disease such as hair color or head size, they would have been unsuccessful. Using increased plasma phenylalanine concentrations as the phenotypic characteristic to define the disease made it much easier to find a genetic marker because the distinction between affected and non-affected individuals was much clearer. This clearer definition of the disease eventually led to the identification of a genetic mutation in the phe-

nylalanine hydroxylase gene as a cause of the disease [9].

The analogy we would like to draw is that hyperglycemia (NIDDM) is a phenotypic characteristic, much like head size or hair color in phenylketonurics, that is quite distant from the underlying pathogenic genotype. There may be many environmental factors and other genetic factors that influence the expression of hyperglycemia and NIDDM given an abnormal genotype. To improve chances of identifying a genetic marker (or markers) of the disease, genetic linkage and association studies should be performed using the most refined definition of the phenotype that is possible. This requires more detailed metabolic studies than simple measurements of plasma glucose concentrations. We have used a variety of clinical and biochemical approaches to better characterize the phenotype of NIDDM in the Pima Indians with the purpose of understanding and determining the etiology of the disease and to identify more refined phenotypes that should increase chances of identifying genetic markers for the disease in pre-diabetics.

PATHOGENESIS OF NIDDM IN PIMA INDIANS AND IDENTIFICATION OF PRE-DIABETIC PHENOTYPES

Cross-sectional studies have established that NIDDM in Pima Indians, as in other population groups, is characterized by four major metabolic abnormalities: obesity, insulin resistance, an abnormal insulin secretory response to glucose, and an increased rate of hepatic glucose production. However, one or more of these abnormalities may be secondary to the disease process itself, and appear after the disease develops, rather than be an abnormality that precedes NIDDM. Only by performing prospective studies can the sequence in which these metabolic abnormalities appear be determined in relation to one another, and in relation to the evolution of impaired glucose tolerance and subsequent development of NIDDM.

A prospective study of a group of non-diabetic Pima Indians was initiated in 1982 to determine which of these four metabolic defects occurs first, and therefore is the most fundamental to the development of NIDDM. The study is still in progress but preliminary analyses suggest the following etiology and pathogenesis of NIDDM in the Pimas (also see Fig. 2). Insulin resistance is the earliest detectable metabolic abnormality among individuals who eventually develop

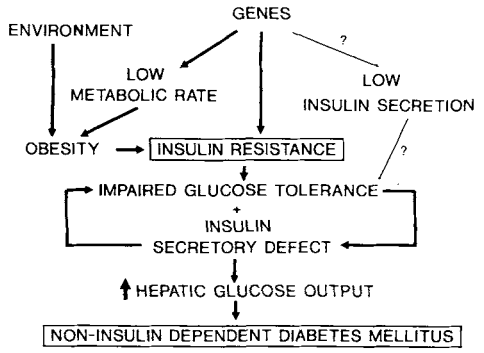

Fig. 2. Hypothesis of the etiologic and pathogenic factors that cause NIDDM in Pima Indians.

NIDDM. Insulin resistance causes impaired glucose tolerance and when, in addition to this abnormality, an insulin secretory defect develops, these two defects result in excessive hepatic glucose output. The overproduction of glucose by the liver, in the presence of insulin resistance and reduced insulin secretion, results in the characteristic marked fasting hyperglycemia of NIDDM. Whether lower insulin secretion also contributes to the development of impaired glucose tolerance is not yet clear. However, it is clear that the transition from impaired glucose tolerance to NIDDM is associated with *secondary* development of an insulin secretory defect, possibly a result of a toxic effect on the beta cell of the prolonged mild hyperglycemia associated with impaired glucose tolerance.

It may be suspected that the only cause of insulin resistance is obesity. This is *clearly not* the case. Insulin resistance is a risk factor for developing NIDDM independent of the effect of obesity. These results from our detailed metabolic studies confirm and extend observations collected in the population survey—that is, that obesity and fasting hyperinsulinemia (as an estimate of insulin resistance) are major risk factors for NIDDM and that fasting hyperinsulinemia is a risk factor independent of the effect of obesity [15,31].

These studies suggest therefore that genetic markers for both insulin resistance and obesity should be sought since markers for these pre-diabetic phenotypic characteristics are likely markers for NIDDM. Since these pre-diabetic metabolic characteristics are "closer" to the putative abnormal genotype(s) than NIDDM itself, using these phenotypic characteristics in linkage studies increases chances that genetic markers will be found.

PRE-DIABETIC PHENOTYPIC CHARACTERISTICS FOR USE IN GENETIC STUDIES

Obesity and/or a Low Metabolic Rate

It is clear from the prospective studies among the Pimas that obesity is a major risk factor for NIDDM and is therefore a pre-diabetic phenotype. The causes of obesity are unknown, but it seems clear that these causes are not gluttony and sloth in all cases.

Obesity shows strong familial aggregation and recent work in Caucasian populations has indicated there are significant genetic determinants of adult body weight [5,35]. Since obesity is a pre-diabetic phenotypic characteristic, if genetic markers for obesity could be found, then they would also be genetic markers for NIDDM. Efforts to identify genetic markers of obesity among the Pimas are currently under way and, if rodent models are any indication, there is likely to be more than one genotype involved in determining the obesity phenotype.

It is possible, however, to further refine the obesity phenotype. Obesity is a result of an imbalance between energy intake and energy expenditure. It is very difficult to quantify energy intake accurately in humans, but technical developments in the last 20 years have made it possible to make prolonged, accurate measurements of rates of energy expenditure in humans. We have used a human respiratory chamber to make measurements of 24-hour energy expenditure (24hEE) in Pima Indians [27]. Rates of 24hEE varied considerably between individuals, independent of individual differences in body composition, age, and sex. In prospective studies, Pimas with a low metabolic rate were more likely to gain weight than Pimas with a high metabolic rate [28]. In addition, 24hEE (independent of body composition, age, and sex) shows strong familial aggregation in the population [8]. Familial aggregation can result from environmental as well as genetic factors. However, Fontaine et al. [10] have reported a higher concordance of metabolic rate among monozygotic twins as compared to dizygotic twins, so that genetic factors are *very* likely to contribute to the familial aggregation of 24hEE. The biochemical and molecular mechanism of a lower metabolic rate among some of the Pimas is not yet known, but recent studies comparing the effect of beta-adrenergic blockade in Caucasians and Pimas suggest the Pimas may have an abnormality in the stimulation of metabolic rate by the sympathetic nervous system [30]. Such an abnormality may lead to a low metabolic rate and be responsible for the observed familial aggregation of energy expenditure.

From these detailed metabolic studies among the Pimas, it is becoming increasingly clear that a low metabolic rate may be a genetically determined phenotype that predisposes to obesity, which in turn increases the risk of developing NIDDM. A low metabolic rate is therefore another candidate pre-diabetic phenotypic characteristic that is being used to search for genetic markers of a predisposition to NIDDM in the Pimas.

Insulin Resistance

Prospective studies have proven that non-diabetic Pimas who are insulin resistant are more likely to get NIDDM than Pimas who are insulin sensitive. It is also very clear that insulin resistance is not entirely due to obesity. Becoming obese may worsen insulin resistance in some individuals, but the relationship between obesity and insulin resistance is much more complex than previously thought.

In non-diabetic Pima Indians, at physiologic plasma insulin concentrations the degree of obesity is non-linearly related to insulin action (Fig. 3A), and at maximally stimulating insulin concentrations the relationship is linear but very weak (Fig. 3B) [17]. More importantly, at either insulin concentration, and at any degree of obesity, there is a wide range in insulin action, demonstrating that obesity explains only a fraction of the observed variance in in vivo insulin action in Pimas. In addition, only a small fraction of the variance in insulin action not due to obesity is due to individual differences in age, degree of physical fitness, or gender [18].

Since individual differences in degree of obesity and physical fitness did not account for more than about one-third of the variance in insulin action in the Pimas, we considered the possible effects of genetic factors. In vivo insulin action shows strong familial aggregation, particularly at maximally stimulating insulin concentrations [8]. Also, the frequency distribution of maximal insulin action is best fit by a mixture of three normal distributions [3] and this is particularly evident among Pimas who were already obese (Fig. 4). These data were consistent with the inheritance of a co-dominantly inherited

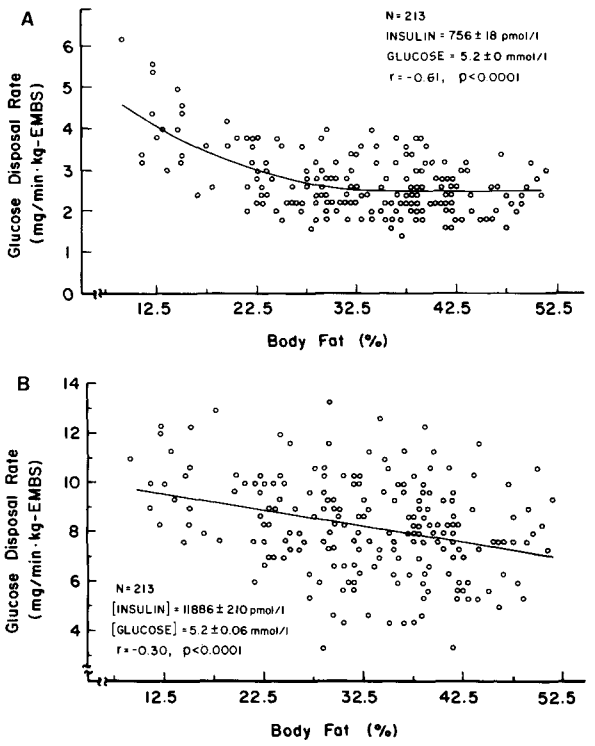

Fig. 3. Relationship between percent body fat and insulin-mediated glucose uptake rates at physiologic (A) and maximally stimulating plasma insulin concentrations (B) in non-diabetic Pima Indians. Insulin-mediated glucose uptake rates were determined using the hyperinsulinemic, euglycemic clamp technique and percent body fat was determined by hydrodensitometry. EMBS, estimated metabolic body size [see reference 17].

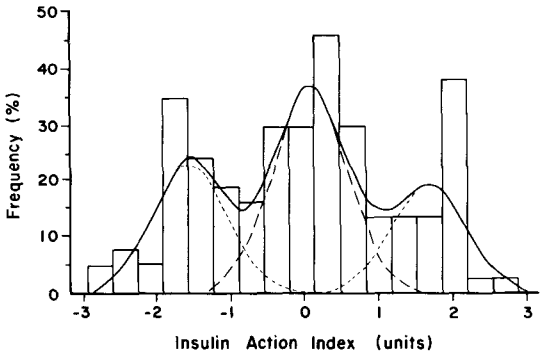

Fig. 4. Frequency distribution of Insulin Action Index, an estimate of insulin action in vivo, in obese (percent body fat ≥ 34.6) non-diabetic Pima Indians. The best fitting model to the data was a mixture of 3 normal distributions determined by maximum-likelihood estimation. Dashed lines represent individual distributions; solid line is the sum of 3 normal distributions, n = 123. Insulin Action Index is a principal component score of fasting plasma insulin concentration and rates of maximal, insulin-mediated glucose uptake rates and is considered the best estimate of in vivo insulin action.

TABLE I. Insulin Action in Skeletal Muscle of Pima Indians*

Normal	Abnormal
Insulin binding	Glycogen synthase
Insulin receptor tyrosine kinase	Protein phosphatase 1
Casein kinase II	cAMP-dependent protein kinase
	Protein tyrosine phosphatase

*See references 4, 12, 13, 19, 21, 23.

autosomal gene as a significant determinant of insulin resistance among the Pimas.

Thus, three features make insulin resistance a promising phenotypic characteristic for genetic association and linkage studies with the purpose of identifying genetic markers for NIDDM: insulin resistance is a risk factor for NIDDM, insulin action in vivo aggregates in families, and insulin action in vivo is trimodally distributed.

It would be expected therefore that if insulin resistance could be defined more precisely at the biochemical level, chances for finding genetic markers would be enhanced, since, at this level, the putative abnormal gene product causing insulin resistance would be more directly related to the gene(s) itself. Since it is known that skeletal muscle is the most important tissue in determining the extent of insulin action in vivo in humans we have studied the effects of insulin on intermediary metabolism in this tissue. Human skeletal muscle tissue is obtained by percutaneous biopsy of the vastus lateralis muscle before, during, and/or after intravenous infusion of insulin during a euglycemic clamp. With this method, several enzyme proteins that have either abnormal basal activity or abnormal responses to insulin have been identified in insulin resistant Pimas [4,12,13,21] (Table I). Other cellular actions of insulin, such as insulin binding, insulin receptor tyrosine kinase activity toward the artificial substrate glu/tyr [23], and activation of casein kinase II [19], have been found to be normal (Table I). The precise biochemical abnormality leading to any of the abnormal enzymatic activities has yet to be identified, so a clearly defined abnormal phenotypic characteristic at the biochemical level has not yet been described. However, it is expected that with further study, insulin resistance will be described at the biochemical level, and this phenotype should offer the greatest promise for

successful linkage studies to identify a genetic marker(s) for NIDDM.

CANDIDATE GENE VS. "REVERSE GENETICS"

In addition to defining which phenotype to use in the search for a genetic marker of NIDDM, there is also the question of which strategy to use. In the absence of a precisely defined, single biochemical abnormality as the cause of obesity, insulin resistance, or NIDDM, what is the quickest and most efficient way to find an abnormal gene that contributes significantly to causing NIDDM? One approach is to work through a list of candidate genes, such as the insulin gene, the insulin receptor gene, etc., and, using a variety of possible techniques, identify an abnormal cDNA gene sequence or abnormal genomic sequence. An alternative is a "reverse genetic" approach as was used to identify genetic markers for Huntington's disease and cystic fibrosis, etc. With this approach strategically located markers on all the human chromosomes are systematically tested for linkage to the selected phenotype with the expectation that a genetic linkage will eventually be found.

There are advantages and disadvantages to each approach. If, from candidate genes, an association is found between a disease phenotype and a particular candidate gene, either by direct sequencing of cDNA or by identification of an associated polymorphism, then not only has a *marker* gene been found, but the *specific* gene has been identified that directly contributes to the occurrence of the disease. This represents an advantage over the "reverse genetic" approach, where a genetic marker of a disease may be identified and proven to be adjacent to the disease locus, but considerable time and effort may then be required to identify the specific abnormal gene directly causing the disease. For example, a genetic marker for Huntington's disease was found on chromosome 4p in 1983, but the specific gene responsible for this linkage has yet to be identified [25].

Conversely, candidate genes are only as valuable as the quality of the candidates. Without a very thorough understanding of the specific biochemical process causing the disease, candidate genes become only poorly educated guesses, which essentially reduces the approach to a haphazard screening of the genome.

Unfortunately, at present, in regard to NIDDM, it is this latter situation in which we find ourselves. Because our understanding of the biochemical mechanisms that lead to the phenotype of NIDDM (or obesity or insulin resistance) is so limited, the candidate gene approach can hardly be distinguished from a haphazard "reverse genetic" approach. In our view, a "reverse genetic" approach, conducted as an orderly screening of the genome, is likely to be the more efficient means in the end.

Bell et al. have already successfully used this approach to locate a genetic marker (adenosine deaminase) on chromosome 20q for maturity onset diabetes of the young (MODY) in a large Caucasian pedigree [2]. Recently, we have found preliminary evidence for genetic linkage between a genetic marker on chromosome 4q and insulin resistance in the Pimas. We are currently trying to confirm this observation using other genetic markers in the same area of the genome.

SUMMARY AND CONCLUSION

More than half the Pima Indians over 35 years of age have NIDDM. Because of this, the Pimas have been the focus of prospective epidemiologic and metabolic studies for over two decades. Based on the results of detailed metabolic studies, we have developed a tentative hypothesis of the etiology and pathogenesis of NIDDM (see Fig. 2) and have identified insulin resistance and a low metabolic rate as pre-diabetic phenotypes. Use of these phenotypes in linkage analyses in Pima families offers the best chance of finding genetic markers for NIDDM since the Pima population is less genetically heterogeneous, and these pre-diabetic phenotypic characteristics are closer to the putative abnormal genotypes that result in the more distant phenotype—NIDDM.

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