Perspectives in Diabetes

Mapping Genes in Diabetes

Genetic Epidemiological Perspective

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Research on mapping diabetes-susceptibility genes is dependent on several factors, including the existence of a single major gene for susceptibility, genetic homogeneity, and the existence of appropriate clinical material. The power to detect susceptibility genes is dependent on the risks in relatives and the distance of genetic markers from the susceptibility genes. For insulin-dependent diabetes mellitus (IDDM), the best-fitting risk models are those with a single major locus with residual polygenic factors. The major locus effect is likely represented by genes in the HLA complex, because specific genotypes have been found to affect IDDM risk significantly. Thus, mapping the remaining polygenic IDDM susceptibility factors—each of small effect—is a difficult and long task. For non-insulin-dependent diabetes mellitus (NIDDM), the likely risk models result in few genes with moderate effect. Models of NIDDM have significant residual polygenic variation remaining, reflecting the importance of multiple loci with small effect, environmental effects, or genetic heterogeneity; however, the prospects for mapping genes that provide at least moderate susceptibility for NIDDM now appear promising. *Diabetes* **39:1315–19, 1990**

The application of molecular genetic strategies to problems of inherited susceptibility to diabetes mellitus has generated considerable enthusiasm. Until recently, mapping disease-susceptibility genes was tedious, time consuming, and often unrewarding. The likelihood of mapping a disease locus to a known genetic marker locus was small because of the limited number of markers available. The genetic markers for these mapping studies were traits (e.g., the ABO blood group) whose lo-

cation, number of alleles, and transmission were known in a population. With the strategy of restriction-fragment–length polymorphisms (RFLPs), many DNA markers have become available (1). Thus, the likelihood of successfully mapping a disease-susceptibility gene has improved dramatically, assuming that the disease is controlled by a gene with a major effect and is genetically homogeneous. For insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), however, these assumptions are of concern. My objective in this perspective is to discuss the features of IDDM and NIDDM that represent challenges to the design of linkage studies and to indicate the implications of these features.

IDDM and NIDDM are actively being investigated by gene-mapping techniques. The pathogenesis of IDDM and that of NIDDM suffer from a common malady—their mode of inheritance is unknown (2,3). The most powerful predictor of risk for diabetes (either IDDM or NIDDM) is being an identical (monozygotic [MZ]) twin of a diabetic person. An important epidemiological finding for IDDM is that the MZ twin concordance rate is significantly <100%, more likely ranging from 25 to 50% (4). The decreased MZ twin concordance rate implies that nonfamilial environmental factors play an important role in defining an individual's susceptibility to IDDM. The random environmental factors may be heterogeneous (prenatal, perinatal, cultural) and may further obscure the mode of genetic transmission. Heterogeneous environmental factors (environmental "triggers," e.g., viruses and exposures to pathogens) could effectively hide single gene effects. Unlike that for IDDM, the MZ twin concordance rate for NIDDM is close to 100%, indicating that the susceptibility is highly familial, with NIDDM determined by genetic or common environmental factors (5).

Estimated risks for diabetes among the relatives of diabetic people from four studies are shown in Fig. 1 (6–9). The degree of genetic relationship (the proportion of genes shared identically by descent between relatives) progresses from tertiary relatives (sharing 12.5% of genes) to secondary relatives (sharing 25% of genes) to first-degree relatives (sharing 50% of genes) to MZ twins (sharing 100% of genes).
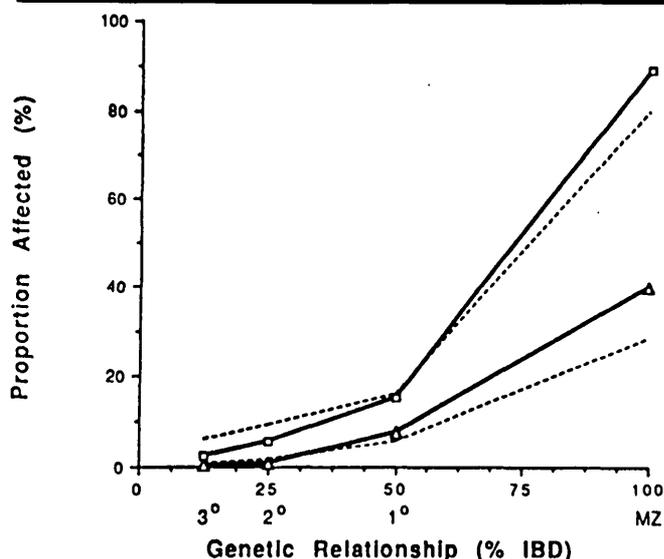
FIG. 1. Lifetime risks for insulin-dependent diabetes mellitus (△) and non-insulin-dependent diabetes mellitus (□) among relatives of diabetic patients. *Solid lines,* observed risk estimates by degree of genetic relationship; *dashed lines,* predicted risks under multifactorial threshold model. MZ, monozygotic twin; 1°, 1st-degree relative; 2°, 2nd-degree relative; 3°, 3rd-degree relative; IBD, identical by descent.

Individual observations of risk to relatives vary from study to study and from population to population. Although there is reasonable agreement on diabetic risk to siblings, the estimates for risks to offspring and second- and third-degree relatives are lacking in confidence. Further studies targeted to these more genetically distant groups are needed. Even though the individual estimates of risk may be disputed, there is a clear relationship between the magnitude of diabetic risk observed and the degree of genetic relationship of the proband. Of interest is the observation that as the degree of relationship decreases from MZ twin to first-degree relatives (full siblings, parents, and offspring) to second-degree relatives to third-degree relatives, the risks for IDDM and NIDDM decrease in a nonlinear fashion. The genetic models that test the hypotheses concerning mode of transmission of IDDM and NIDDM are evaluated by their ability to match these epidemiological findings.

A hypothesis that is often speculated for IDDM and NIDDM is that a single locus is responsible for genetic susceptibility. This single-locus model is attractive for several reasons, the primary one being that a single locus has a high probability of being mapped to a chromosomal location. Although genetic susceptibility to IDDM is strongly influenced by genes in the HLA region of human chromosome 6, not all of the susceptibility is accounted for by this one region. This can be seen by the observation that 55% of trait-concordant sibling pairs (both siblings have IDDM) will have the same HLA region genes (haplotypes), 40% of the sibling pairs will have only one haplotype in common, and 5% of the sibling pairs will have no HLA haplotypes in common (10). The failure of the single-locus model for IDDM susceptibility is often reconciled by invoking reduced penetrance (those subjects with the disease genotype not developing IDDM) or by allowing for sporadic subjects (those without the disease genotype developing IDDM). The single-locus models fail to account for the nonlinear nature of familial risk for IDDM and NIDDM.

In IDDM, the risk to siblings of a proband is not significantly different from the risk to offspring (9). Because the difference in genetic expectation for these classes of relatives involves dominance, the similarity in risk implies that the genetic variation for IDDM is additive. Under a strictly additive single-locus model, the risk to relatives of a proband is a linear function of the population prevalence, the additive genetic variance, and the probability that the proband and relative share an allele identical by descent (11). This linear prediction of risk of IDDM to relatives is in sharp contrast to the nonlinear estimates in Fig. 1. The degree of reduced penetrance required for a single locus to account for the low IDDM rates in second- and third-degree relatives is difficult to reconcile with the observed MZ twin concordance rate. Similar reasoning indicates that a single-locus model is even less likely for NIDDM.

Multiple-locus models, defined by many genes, each with small and additive effects, can approach the nonlinear form of the risks to relatives for both IDDM and NIDDM. In a multifactorial threshold model, the genetic factors combine in an additive fashion to determine the susceptibility to diabetes. Each component (genetic and environmental) contributes to an underlying scale of liability. Once the liability exceeds a threshold value, the disease is assumed to occur. Based on the prevalence of disease in the population and the MZ twin concordance rate, the heritability of liability for IDDM and NIDDM can be calculated (12). With a population prevalence of 4/1000 and an MZ twin concordance rate of 40%, the heritability of IDDM liability was estimated at 80%. Similarly, the heritability of liability for NIDDM was estimated at 70%. With these values, the expected risks for first-degree, second-degree, and third-degree relatives were calculated (Fig. 1). Although the predicted MZ twin concordance rate is lower than that observed for IDDM and NIDDM, the multifactorial threshold model produces a relative risk function consistent with the observed nonlinear shape.

Although a multifactorial source of genetic liability to IDDM and NIDDM is troubling for gene mapping, there is no need to imply that multifactorial inheritance implies hundreds of loci. Instead, a more likely outcome is that IDDM and NIDDM are caused by a limited number of polygenes or a polygene whose effect is large compared with other polygenes. If genetic heterogeneity exists so that some cases are caused by "true" polygenes and others are caused by a "major" polygene, the effort to map diabetes-susceptibility genes becomes increasingly difficult.

Although multiple loci may influence diabetes susceptibility, there is hope that single genes may be mapped. Previous strategies used in mapping disease-susceptibility genes have centered on the choice of clinical material and genetic markers. Clinical material can consist of individuals (probands), sibling pairs, families, or pedigrees. Much of the flexibility in choice depends on the features of the disease, including age-at-onset distribution, mode of inheritance, and genetic heterogeneity.

Recent successes of human gene mapping have used a strategy termed *reverse genetics.* This approach uses a large panel of informative (polymorphic) genetic markers (e.g., RFLPs) in many families with the disease of interest. The mapping of the disease locus reduces to finding in families genetic markers that tend to segregate with the disease. Once the disease locus has been isolated to a specific chro-

mosomal region, genetic markers that are positioned on both sides of the disease locus are found. The region within the markers can then be searched for the disease gene. The mapping of the genes for Huntington's chorea to chromosome 4 (13), cystic fibrosis to chromosome 7 (14), and neurofibromatosis to chromosome 17 (15) have had a major impact on risk prediction, counseling, and, ultimately, gene therapy and prospects for prevention. However, these disorders have a common feature not shared by IDDM or NIDDM. Each mapped disease is caused by one gene, with little evidence for genetic heterogeneity. Thus, the obvious strategy of investigating large pedigrees, which is employed by reverse genetics, may be ineffective in the light of multiple genes or genetic heterogeneity.

IDDM has an early onset, but the reduced recurrence in relatives makes it difficult to obtain pedigrees with diabetic siblings or diabetic family members in several generations. NIDDM has a late onset, so the pedigrees with parent-offspring transmission often occur only after the affected parent is deceased. Because the genetic mechanism is unknown for IDDM and NIDDM, standard likelihood methods may not be able to detect and estimate linkage relationships between the loci controlling the presence of disease (IDDM or NIDDM) and the marker loci (16). Because affected parent-offspring relationships are difficult to ascertain for IDDM and NIDDM, the most closely related clinical material available for investigation of genetic susceptibility involves siblings.

One approach to the search for genetic factors that contribute to disease susceptibility is to demonstrate an association between a genetic marker and the disease. With a random sample of unrelated diabetic and control subjects, a marker that is increased in frequency in diabetic versus control would appear to be a candidate for a disease-susceptibility locus. However, the marker should not have a selective effect on the individual, because an association between the selective effect and the disease would result in a spurious association with the marker (17). Although RFLPs should not have selective effects, candidate genes (by their nature of having some perceived importance in the pathway of disease) may have a selective effect.

An early approach to the detection of linkage based on a model-independent sibling relationship was the Haseman and Elston method (18), which was restricted to a quantitative trait (e.g., glucose level) rather than disease status. Extension of the method to disease traits corresponds to testing whether the proportion of marker alleles identical by descent (IBD) in the sibling pairs is the same for trait-concordant and -discordant pairs. Thus, the average proportion of marker genes IBD for trait-concordant sibling pairs (NIDDM-NIDDM and unaffected-unaffected) should be >50% (the expected value under the assumption of no linkage). Similarly, the average proportion of marker genes IBD for trait-discordant sibling pairs (NIDDM-unaffected) should be <50%. Although the method of detecting linkage is focused on sibling pairs, other pairs of relatives could be included in the analysis.

The inclusion of other genetic relationships in a model-independent linkage analysis was recently proposed as a means to evaluate genetically complex traits (19–22). The parameter λ_R is used to estimate the risk ratio for a relative of an affected individual compared with the population prevalence. For a single locus without dominance, these ratios

can be estimated and compared with the observed (average) risks to relatives. For IDDM, the risks for siblings and offspring of IDDM probands are similar in magnitude. For this article, lifetime risk is 0.4% and the risk ratio in siblings and offspring is 8%, yielding $\lambda_S = \lambda_O = \lambda_1 = 20$, somewhat higher than that previously estimated (23). Observed risk ratio for MZ twins of IDDM probands is $\lambda_M = 100$, and that for second-degree relatives is $\lambda_2 = 3$. For NIDDM, lifetime risk is 8%, the risk ratio for MZ twins of NIDDM probands is $\lambda_M = 10$, that for first-degree relatives of NIDDM probands is $\lambda_1 = 3.5$, and that for second-degree relatives of NIDDM probands is $\lambda_2 = 1.5$. Given more accurate estimates of risk to relatives from epidemiological studies, these risk ratios could change significantly, which could affect the fit to the proposed genetic models.

For a single-locus model for IDDM, the risk ratio for an MZ twin of an IDDM proband (λ_M) was predicted to be 39, and the risk ratio for second-degree relatives (λ_2) of an IDDM proband was predicted to be 10.5 (Table 1). The NIDDM risk ratio for an MZ twin of an NIDDM proband was predicted to be $\lambda_M = 6$, and the risk ratio for a second-degree relative of an NIDDM proband was predicted to be $\lambda_2 = 2.3$. Thus, with a single-locus model for IDDM and NIDDM, the second-degree relative risk ratios are overestimated, and the MZ twin risk ratios are underestimated. This pattern of estimation suggests that multiple loci or epistatic effects are required to mimic the observed nonlinear risk function in relatives, reflecting a more rapid decrease in risk with increasing genetic distance.

Several multiplicative (multiple-locus) models can be tested for improved fit to the observed risk relationships. A model with an infinite number of loci, each with a small effect (a polygenic model), would predict risk ratios for MZ twins of IDDM probands of $\lambda_M = 400$ and risk ratios for second-degree relatives of IDDM probands of $\lambda_2 = 4.5$. Risk ratios for third-degree relatives of IDDM probands were also predicted to be high ($\lambda_3 = 2.1$). For the polygenic models to

TABLE 1
Predictive risk models for insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) by degree of genetic relationship

Model	IDDM risk ratio			NIDDM risk ratio		
	λ_M	λ_1	λ_2	λ_M	λ_1	λ_2
Observed	100	20	3.0	10.0	3.5	1.5
One major locus	39	20	10.5	6.0	3.5	2.3
Polygenes	400	20	4.5	12.3	3.5	1.9
Two loci	63	20	7.4	7.4	3.5	2.1
Mixed	94	20	7.1	10.0	3.5	1.9
Multiplicative 2a	96	20	6.5	10.3	3.5	1.9
Multiplicative 2b	99	20	6.4	10.1	3.5	1.9

λ_M, Risk ratio to monozygotic twin of diabetic patient; λ_1, risk ratio to 1st-degree relative of diabetic patient; λ_2, risk ratio to 2nd-degree relative of diabetic patient. Two loci: IDDM, $\lambda_{11} = \lambda_{12} = 4.5$; NIDDM, $\lambda_{11} = \lambda_{12} = 1.9$. Mixed: IDDM, major gene with $\lambda_1 = 8$ and polygenes with $\lambda_* = 2.5$; NIDDM, major gene with $\lambda_1 = 1.75$ and polygenes with $\lambda_* = 2.0$. Multiplicative 2a: IDDM, 2 major genes with $\lambda_{11} = \lambda_{12} = 3.5$ and polygenes with $\lambda_* = 1.6$; NIDDM, 2 major genes with $\lambda_{11} = \lambda_{12} = 1.4$ and polygenes with $\lambda_* = 1.8$. Multiplicative 2b: IDDM, 2 major genes with $\lambda_{11} = 5$ and $\lambda_{12} = 2.2$ and polygenes with $\lambda_* = 1.8$; NIDDM, 2 major genes with $\lambda_{11} = 1.8$ and $\lambda_{12} = 1.1$ and polygenes with $\lambda_* = 1.8$.

predict the risk ratios for second- and third-degree relatives accurately, the risk ratio for an MZ twin of an IDDM proband would be seriously overestimated. For NIDDM, the polygenic model would predict risk ratios for MZ twins of NIDDM probands of λ_M = 12.3 and risk ratios for second-degree relatives of NIDDM probands of λ_2 = 1.9. The risk ratio for third-degree relatives of NIDDM was predicted to be λ_3 = 1.4. Thus, the polygenic model slightly overpredicts the MZ and second-degree risk ratios for NIDDM.

Multiplicative models incorporate interaction of multiple factors, so risk ratios are the products of the individual factors from the contributing loci. This situation would be representative of an epistatic effect, in which specific combinations of factors are required for the disease to be present. In the model of IDDM and NIDDM, a two-locus multiplicative model always underestimates the MZ risk ratios and overestimates the risk ratios for second-degree relatives.

A combination of major genes and polygenes would likely provide a closer fit to the observed risk data. When a single major locus with a large effect is combined with residual polygenes, denoted by mixed in Table 1, the predictions for NIDDM and IDDM are more consistent with the observed risks, with only the risks to second-degree relatives overestimated. However, the major locus for NIDDM has a proportionate risk less than that for the polygenic component. Thus, the major locus in this mixed model for NIDDM has a small effect. The risk ratio of λ_1 = 2.0 for the major locus indicates that the genotype at this locus would contribute only a twofold increased risk to first-degree relatives over that of the general population. The magnitude of effect of the NIDDM major locus in the mixed model contrasts sharply with the major locus for IDDM (λ_1 = 8.0), which provides an eightfold increased risk over the population rate.

Other mixed models used two major genes in multiplicative action with polygenes. The models denoted by multiplicative 2a in Table 1 represent two major genes of equal effect with polygenes. For IDDM, each major locus contributed 3.5 to the risk ratio (λ_1 = 3.5), with residual polygenic variation contributing $\lambda_{1'}$ = 1.6. For NIDDM, each major locus contributed λ_1 = 1.4 to the risk ratio, with residual polygenes contributing $\lambda_{1'}$ = 1.8. The risk ratios for MZ twins for both IDDM and NIDDM are well predicted (Table 1). The risk ratios for second-degree relatives of IDDM and NIDDM probands are overpredicted, with the more serious discrepancy in IDDM risk. Note, however, that each of the major loci for NIDDM contributes less to the risk ratio than the polygenic component.

Models of two major loci of different effect and polygenes contributing to the risk ratio are denoted by multiplicative 2b in Table 1. The risk ratio for IDDM is composed of one locus with a proportionately larger effect (λ_1 = 5.0) than a second locus, with an effect similar to that of the polygenic contribution (λ_1 = 2.2 and $\lambda_{1'}$ = 1.8, respectively). The risks for second-degree relatives of IDDM and NIDDM probands continue to deviate from the observed. For NIDDM, the two major loci have significantly lesser effects than the major loci for IDDM.

Based on these analyses, the inheritance of IDDM appears consistent with a single major locus providing significant susceptibility but requiring many contributing factors with equal and additive effects. Any second major locus would

not have a significant contribution to the risk compared with that contributed by polygenes. This result implies that if the genes in the HLA complex constitute the major IDDM susceptibility factor, the remaining contributors to IDDM risk to relatives are small in effect. The approaches to mapping polygenes in humans are still in the formative stages and center on the availability of a human gene map with genetic markers spaced an average of 1–2 centi-Morgans (1–2 million base pairs) apart. In view of the important role of environmental risk factors (reflected by the low MZ twin concordance rate), which may mimic genetic transmission of IDDM, these additional genetic factors will likely be difficult to map.

In contrast, the models of risk to relatives of NIDDM patients appear compatible with a single locus with polygenes or a few multiplicative loci with polygenes. Although the major effects modeled for NIDDM susceptibility are not as large as those found for IDDM susceptibility, the support for these models is promising. It is surprising that NIDDM, which is acknowledged to be clinically heterogeneous with multiple pathways likely leading to disease, may require only a few loci to provide a significant risk to relatives. Thus, the task of mapping a series of single genes with moderate effects on NIDDM susceptibility appears feasible.

Although many investigators are now searching for the elusive diabetes-susceptibility genes (for both IDDM and NIDDM), a few cautionary notes are required. The suggestion of modes of inheritance depended on the assumptions of risks to relatives of diabetic patients. As previously noted, the risk estimates for second- and third-degree relatives need to be determined with greater precision. Because most models adequately predict the MZ risk ratios based on the first-degree risk ratios, the best models would accurately predict these additional risks. All models failed to predict the decreased risk in second-degree relatives adequately. In addition, genetic heterogeneity may obscure the search for major genes, because different loci would lead to disease in different families. It has been previously shown, however, that use of more distant relatives (such as affected cousins) would provide more power to detect linkage than use of affected siblings (20). Further complications arise from the important effect environmental factors have on diabetic susceptibility. For IDDM, the MZ twin concordance rate is substantially <100%, which implies that many patients and relatives may have IDDM because of the presence of an unidentified environmental factor. The greater the environmental contribution to susceptibility, the greater the difficulty in mapping. This difficulty can be envisioned by having two affected relatives, one with IDDM from genetic risk and one with IDDM from environmental risk. Because the MZ twin concordance rate for NIDDM is ~100%, the effects of environment may not be as great a concern.

Strategies need to be developed for mapping diabetes-susceptibility genes. The usual approach of mapping genes by linkage analysis in nuclear families by the lod-score method may not be effective. The power for the affected-relative–pairs approach to detect linkage is dependent only on the magnitude of risk λ_R and the recombination fraction θ. Risch (20) showed that for λ_s = 2 and tight linkage (θ = 0), a sample size of 200 pairs would be sufficient to detect linkage with 80% power. This sample size would be appro-

priate for many models of NIDDM susceptibility. As the risk ratio increases, so does the evidence for major genes. Thus, for $\lambda_s = 5$, only 60 pairs of affected siblings are needed to detect linkage with 80% power. The genetic relationships needed for sampling in gene mapping depend on the recombination fraction and the magnitude of λ_R. In some cases, it may be preferable to use affected grandparent-grandchild or cousin pairs rather than sibling pairs.

In summary, substantial progress has been made in defining genetic and epidemiological risk factors for IDDM and NIDDM. Both diseases have modes of inheritance that have yet to be adequately resolved, even though a major contributor to genetic risk for IDDM has been identified (HLA). The strategy for detecting additional IDDM-susceptibility genes is complicated by the presence of environmental effects and polygenes. Studies of genetic markers of NIDDM, which are considerably more prevalent in the population, have failed to identify a single major gene that contributes substantially to genetic risk. However, compatible risk models for NIDDM contain few genes with moderate effect with residual polygenic variation. Thus, even in the presence of potential heterogeneity and multiple pathways to NIDDM, the prospects for mapping NIDDM-susceptibility genes appear promising.

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