GENETIC MARKERS

1. Use

A. Diagnosis/Screening

a. Type 1 diabetes

Recommendation: Routine measurement of genetic markers is not of value at this time for the diagnosis or management of patients with type 1 diabetes. For selected diabetic syndromes, valuable information can be obtained with definition of diabetes-associated mutations.

Level of evidence: E

Genetic markers are currently of limited clinical value in the evaluation and management of patients with diabetes. However, they hold promise for the future. For immune-mediated (type 1A) diabetes (IMD), HLA typing can be useful to indicate absolute risk of diabetes (see Table 6), as extended by insulin (*INS*) gene typing (and in some populations by CTLA-4 gene typing), and can assist in assigning a probability of the diagnosis of IMD to diabetes of uncertain etiology (196). As indicated below, HLA-DR/DQ typing can be useful to indicate modified risk of IMD in persons with positive islet cell autoantibodies, since protective alleles do not prevent the appearance of islet cell autoantibodies (most often as single autoantibodies), but do protect against clinical diabetes. Typing of class II major histocompatibility antigens or HLA-DRB1,-DQA1 and -DQB1 is not diagnostic for IMD. However, some haplotypes form susceptibility, while others provide significant protection. Thus, HLA-DR/DQ typing can be used only to increase or decrease the probability of IMD presentation, and cannot be recommended for routine clinical diagnosis or classification (197).

Table 6: Lifetime Risk of Type 1 Diabetes in First-Degree Relatives*
(proband diagnosed before age 20)

Relative	Risk (%)
Parents	2.2 ± 0.6%
Children	5.6 ± 2.8%
Siblings	6.9 ± 1.3%
HLA-non-identical sib	1.2%
HLA-haploidentical sib	4.9%
HLA-identical sib	15.9%
Identical twin	30-40%
General population	0.3%

*From Harrison (205)

It is possible to screen newborn children to identify those at increased risk of developing IMD (198, 199). This strategy cannot be recommended until there is a proven intervention available to delay or prevent the disease (200). The rationale for the approach is thus placed below under emerging considerations.

b. Type 2 diabetes and MODY

Recommendation: There is no role for routine genetic testing in patients with type 2 diabetes. These studies should be confined to the research setting and evaluation of specific syndromes.

Level of evidence: E

Type 2 diabetes: Fewer than 5% of patients with type 2 diabetes have been resolved on a molecular genetic basis and, not surprisingly, most of these have an autosomal dominant form of the disease or very high degrees of insulin resistance. Type 2 diabetes is a heterogenous polygenic disease with both resistance to the action of insulin and defective insulin secretion (3, 4). Multiple genetic factors interact with exogenous influences (e.g., environmental factors such as obesity) to produce the phenotype. Identification of the affected genes is therefore highly complex.

MODY: Mutation detection for maturity onset diabetes of youth (MODY) patients and their relatives is technically feasible. However, due to the high cost of establishing a facility to detect mutations and the high level of technical skill required for analysis, few laboratories perform these assays. As direct automated sequencing of genes becomes standard, it is likely that detection of specific diabetes mutations will become more common.

B. Monitoring/Prognosis

Although genetic screening may provide information about prognosis and could be useful for genetic counseling, genotype may not correlate with the phenotype. In addition to environmental factors, interactions among multiple quantitative trait loci expressions may be involved. Genetic identification of a defined MODY will have value for anticipating the prognosis.

2. Rationale

The HLA system, which has a fundamental role in the adaptive immune response, exhibits considerable genetic complexity. The HLA complex on chromosome 6 contains class I and II genes that code for several polypeptide chains (201). The major (classic) class I genes are *HLA-A, -B* and *-C*. The loci of class II genes are designated by three letters: the first (*D*) indicates the class, the second (*M, O, P, Q* or *R*) the family and the third (*A* or *B*) the chain. Both classes of molecules are heterodimers; class I consists of an α chain and β2-microglobulin, while class II has α and β chains. The function of the HLA molecules is to present short peptides, derived from pathogens, to T cells to initiate the adaptive immune response (201). Genetic studies have revealed an association between certain HLA alleles and autoimmune diseases. These diseases include, but are not confined to, ankylosing spondylitis, celiac disease, Addison's disease and type 1 diabetes (201).

Genetic testing for syndromic forms of diabetes is the same as that for the underlying syndrome itself (1). Such diabetes may be secondary to the obesity associated with Prader-Willi syndrome, which maps to chromosome 15q, or to the absence of adipose tissue inherent to recessive Seip-Berardinelli syndrome of generalized lipodystrophy mapping to chromosome 9q34 (1, 202). There are over 60 distinct genetic disorders associated with glucose intolerance or frank diabetes. Most forms of type 2 diabetes (which are usually strongly familial) will probably be understood in defined

genetic terms, but this is far from realized at present. Some genes for MODY have been identified, but there are large numbers of individual mutants. Persons at risk within MODY pedigrees can be identified through genetic means. Depending on the specific MODY mutation, the disease can be mild (e.g., glucokinase mutation) and not usually associated with long term complications of diabetes or as severe as typical type 1 diabetes [e.g., hepatocyte nuclear factor (HNF) mutations] (203). The interest in the genetics of MODY is the hope that insight will be obtained into type 2 diabetes. (Note that MODY is not a form of type 2 diabetes.)

Five different MODYs have been identified. MODY-1, 3, 4, and 5 all result from mutations in the genes encoding transcription factors that regulate the expression of genes in pancreatic β cells. These genes are hepatocyte nuclear factor-4α (*HNF-4*α) in MODY-1, *HNF-1*α in MODY-3, *HNF-1*β in MODY-5, and insulin promoter factor-1 (*IPF-1*) in MODY-4. It has been shown that homozygous mutations of the *IPF-1* gene leads to pancreatic agenesis and that heterozygous mutations of *IPF-1* genes results in MODY-4 (202). The modes of action of the HNF lesions in MODY is still not clear. It is likely that mutation in *HNF-1*α*, 1*β*, and 4*α cause diabetes because they impair insulin secretion. MODY-2 is caused by mutations in the glucokinase gene. The product of the gene is an essential enzyme in the glucose-sensing mechanism of the b cells, and mutations in this gene lead to partial deficiencies of insulin secretion.

3. Analytical Considerations

A detailed review of analytical issues will not be attempted here, since genetic testing for diabetes outside of a research setting is currently not recommended for clinical care. Serological HLA typing should be replaced by molecular methods, such as sequence specific priming, since antibodies with a mixture of specificities and cross reactivities have been estimated to give inaccurate results in approximately 15% of typings.

A. Preanalytical

Detection of mutations is performed using genomic DNA extracted from peripheral blood leukocytes. Blood samples should be drawn into test tubes containing EDTA and the DNA preparations should be harvested within 3 days; longer periods both lower the yield and degrade the quality of the DNA obtained. Genomic DNA can be isolated from fresh or frozen whole blood by lysis, digestion with proteinase K, extraction with phenol, and then dialysis. The average yield is 100 to 200 μg DNA from 10 ml of whole blood. DNA samples are best kept at –80˚ C in Tris-EDTA solution, where the integrity of the sample lasts virtually indefinitely.

B. Analytical

Methods for the detection of mutations differ for different types of mutation. The MODYs have substitution, deletion or insertion of nucleotides in the coding region of the genes. These are detected by PCR. The detailed protocols for the detection of specific mutations are beyond the scope of this review.

4. Emerging considerations

To screen for the propensity for IMD in general populations, *HLA-D* genes are the most important, contributing as much as 50% of the genetic susceptibility (196). *HLA-DQ* genes appear to be central to the HLA associated risk of IMD, albeit *HLA-DR* genes may be independently involved [for review, see (204, 205)]. The heterodimeric proteins that are expressed on antigen presenting cells, B lymphocytes, platelets and activated T cells, but not other somatic cells, are composed of cis and trans complementated α- and β-chain heterodimers. Thus, in any individual four possible DQ dimers are encoded. Positive risks for IMD are associated with a chains that have an arginine at residue 52,

and β chains that lack an aspartic acid at residue 57. Persons at the highest genetic risk for IMD are those in whom all four DQ combinations meet this criterion. Thus, persons heterozygous for *HLA-DRB1*04 -DQA1*0301-DQB1*0302* and *DRB1*03-DQA1*0501-DQB1*0201* are the most susceptible, with an absolute life-time risk of IMD in the general population of about 1:12. Persons who are protected from IMD are those with *DRB1*15-DQA1*0201-DQB1*0602* (Asp 57+) haplotypes in particular (206), albeit those with *DRB1*11* or **04* who also have *DQB1*0301* (Asp 57 +) are protected. HLA-DR is also involved in susceptibility to IMD in that the *B1*0401* and **0405* subtypes of *DRB1*04* are susceptible, while the **0403* and **0406* subtypes are protective, even when found in HLA haplotypes of the susceptible *DQA1*0301-DQB1*0302*. DR molecules are heterodimers also, however the DRα chain is invariant in all persons. Additional DRβ chains (B3, B4 and B5) are not important.

Class II MHC is involved in antigen presentation to CD4 helper cells, and the above associations are likely to be explained by defective affinities to islet cell antigenic peptides, leading to persistence of T helper cells which escape thymic ablation. Class I HLA is also implicated in IMD. Multiple non-HLA loci also contribute to susceptibility to type 1 diabetes (204). For example, the variable nucleotide tandem repeat (VNTR) upstream from the insulin (INS) gene on chromosome 11q is also useful for predicting the development of IMD, with alleles with the longest VNTR having protective effects. Typing newborn infants for both *HLA-DR/DQ*, and to a lesser degree the *INS* gene, results in prediction of IMD to better than 1:10 in the general population. The risk of IMD in HLA-identical siblings of a proband with IMD is 1:4, while siblings who have HLA-haplotype identity have a 1:12 risk and those with no shared haplotype a 1:100 risk (205). The numerous other putative genomic intervals suggested to be linked to IMD remain to be confirmed in multiple data sets, and discussion of these is outside the scope of the article. The sequencing of the human genome and the formation of consortia should result in advances in the identification of the genetic bases for both type 1 and type 2 diabetes. This progress should ultimately result in family counseling, prognostic information and the selection of optimal treatment (202, 207) .