I. OVERVIEW

A. INTRODUCTION

This study will establish the Type 1 Diabetes Genetics Consortium (T1DGC). The goal of the Consortium is to organize international efforts to identify genes that determine an individual's risk of type 1 diabetes. Progress towards this goal, worldwide, has been limited by a lack of sufficient clinical and genetic resources. The power of larger collections of families to map type 1 diabetes genes has been demonstrated in a pilot study. The creation of a resource base of well-characterized families from multiple ethnic groups will facilitate the localization and characterization of type 1 diabetes genes that determine disease risk. Statistical genetic analyses will be performed on appropriately powered data sets to identify regions linked to type 1 diabetes and determine how these linked regions act and potentially interact. Building upon these Consortium resources, members and collaborators of the Consortium will undertake positional cloning to identify individual genes that determine susceptibility or protection.

Previously, the search for common, complex disease genes (including those that contribute to type 1 diabetes susceptibility) has been limited by inadequate family sample size (insufficient power) to detect genes of modest statistical effects, but important biological significance, using linkage approaches. In type 1 diabetes, the HLA region is well established as containing the major determinant(s) of susceptibility. Based upon current analyses of completed genome screens, non-HLA region genes may individually contribute relatively smaller increments in genetic risk. For example, the insulin promoter polymorphism, the only other generally accepted genetic contributor to type 1 diabetes risk has an odds ratio (OR) ~ 3 but an estimated sibling genetic risk ratio (λ_S) of only 1.12. Other locus-specific effects, excluding HLA, range from 1.05-1.3. Power analyses suggest that ~4,300 affected sib-pair families will be required to achieve 80% power to detect a linkage with $p < 2.2 \times 10^{-5}$ at these levels of risk.

B. BACKGROUND AND SIGNIFICANCE

Type 1 diabetes is characterized by autoimmune destruction of the pancreatic ß cells. As a result, there is a complete dependence upon exogenous insulin in order to regulate blood glucose levels. Type 1 diabetes is the third most prevalent chronic disease of childhood, affecting 0.3% of the general population by age 20 years and a lifetime risk of nearly 1% (1). It is estimated that approximately 1.4 million persons in the U.S. (10-20 million people worldwide) have type 1 diabetes (2-3). In most cases, a pre-clinical period marked by the presence of autoantibodies to pancreatic ß-cell antigens (GAD_{65}, insulin or IA-2_{ic}) precedes the onset of hyperglycemia. This pre-clinical period provides a theoretical opportunity for prevention in those recognized as "susceptible."

The etiology of type 1 diabetes is unknown, but it is recognized to be due to both genetic and environmental determinants (4). Although type 1 diabetes is responsible for a major reduction in quality of life for those with the disease, the increase in morbidity and mortality resulting from type 1 diabetes is the result of the subsequent complications. Individuals with type 1 diabetes are at great risk of disease in the small vessels that lead to nephropathy, retinopathy and neuropathy. Although the onset of these complications may be delayed by

improved glucose control, there is mounting evidence that risk of the complications of type 1 diabetes may also be mediated in part by genetic factors (5). While deterioration of the small vessels due to type 1 diabetes may result in kidney failure, blindness and amputation, much of the mortality of type 1 diabetes is due to its effect on large vessels (6). Type 1 diabetes has been recognized as a consistent, independent risk factor for disease of the coronary arteries (*e.g.*, heart attack) and cerebral arteries (*e.g.*, stroke). Thus, knowledge of the genetic basis for type 1 diabetes will allow targeted studies to be performed on an epidemiologic basis to determine those factors that initiate the autoimmune cascade in genetically susceptible individuals. In this manner, appropriate interventions can be developed to block the autoimmune process from beginning or progressing and, in effect, lead to disease prevention.

C.   GENETIC BASIS OF TYPE 1 DIABETES

The genetic basis of type 1 diabetes is complex and likely to be due to genes of both large and small effect. There have been numerous studies investigating genetic susceptibility loci, using both case-control and family study designs. Early studies of disease concordance using twin designs reported higher monozygotic (MZ) than dizygotic (DZ) rates, with MZ rates approaching 50% (7-8). These early studies were likely biased; however, as recruitment of the twins was through advertisement and solicitation, so that affected concordant pairs were more likely to participate than discordant pairs. Population-based twin studies confirmed the increased concordance in MZ pairs, but with the concordance of 30%-40%, and the concordance in DZ pairs only 5%-10% (9-10). Based upon the results of twin studies, susceptibility to type 1 diabetes is determined by genetic risk factors, but less than 50% of the risk is due to the effects of genes. Studies of first and second-degree relatives also support familial aggregation of type 1 diabetes (11-13).

1.   The Human Major Histocompatibility Complex (MHC) and Type 1 Diabetes

The statistical association between human leukocyte antigen (HLA) B15 and diabetes was first shown by Nerup and colleagues (14), who also were the first to demonstrate that the association between HLA (B8 and B15) and diabetes was specifically with type 1 diabetes. Based upon a number of studies that took place in Caucasian populations, the HLA association with type 1 diabetes was rapidly confirmed (15-17). Continued examination of the region containing HLA genes identified extensive genetic complexity, with multiple genes in linkage disequilibrium clustered in a narrow (~ 6 Mb) physical region. The original association between type 1 diabetes and HLA was expanded to include the A1-B8 and A2-B15 (class I) haplotypes, suggesting that multiple HLA determinants could contribute to type 1 diabetes susceptibility. An alternative explanation was that there were genes in the region (not HLA itself) that contributed to susceptibility. Further research by the Copenhagen group (18) identified alleles of the DR locus (class II), specifically DR3 (A1-B8-DR3) and DR4 (A2-B15-DR4). From this background, much of the remaining twenty years of research has focused on the role of the HLA complex in type 1 diabetes risk. The primary findings during this period are (a) HLA is not the only type 1 diabetes genetic risk factor; (b) both HLA class I and class II loci are important factors; and (c) HLA class II loci (DR, DQ, DP) appear to be stronger contributors to type 1 diabetes risk than class I (A, B, C) loci (19-23).

Two loci in the HLA class II complex (HLA-DRB1 and HLA-DRQ1) have been shown to represent the major determinants of this region, although other class II (HLA-DPB1) and class I (HLA-A, HLA-B and others) loci may contribute to susceptibility (21-22). It has been suggested that genes in the HLA region may contribute up to 50% of the total genetic risk for type 1 diabetes, although the effect of HLA may be more than simple increase of risk (20, 24). While there may be multiple genes that contribute to risk of type 1 diabetes, the HLA region (*IDDM1*), including the HLA-DR and DQ genes, is the only major genetic determinant. Although published linkage studies may be under-powered to detect effects such as *INS*/*IDDM2* (with OR ~3 and λs ~ 1.12), nearly all studies have at least 99% power to detect HLA/*IDDM1* effects (with λs ~ 3). Nearly 95% of type 1 diabetics has either the DRB1*03, DQB1*0201 or the DRB1*04, DQB1*0302 haplotype. While the heterozygote (DRB1*0301, DQB1*0201/ DRB1*04, DQB1*0302) is present in only 2% of the general population, it is occurs in 30-40% of type 1 diabetic patients in most, but not all, populations (25-26). Even though the role of HLA has been suggested since 1975 and much effort has been spent in dissecting the complexity of the HLA region, much remains to be learned about the manner in which HLA contributes to risk and its interaction with other risk loci. The cost and logistics of a large-scale HLA typing have hampered such studies. In the T1DGC, we propose to HLA genotype all family members who undergo the genome screen to allow for complete use of these important genetic data in all analyses. The Consortium will then facilitate the identification of the non-class II loci using single nucleotide polymorphism (SNP) association analyses conditional on DR/DQ/DP genotype.

It has been shown that studies of disease-associated haplotypes in diverse (African or Asian) or isolated (Sardinia or Finland) populations can aid in fine mapping disease variants from the "trans-racial" comparison of haplotypes that are associated and not associated with disease (27-29). This approach has been pioneered in the study of HLA and type 1 diabetes. The presence of a "true" etiologic variant should correlate with susceptibility (or protection) independent of the adjacent haplotype background. This feature, of course, is complicated in the major histocompatibility complex (MHC), as both DRB1 and DQB1/DQA1 are associated with disease, each with similar function in antigen presentation and immunoregulation. However, trans-racial mapping was able to demonstrate that each of the three primary class II loci (DRB1, DQB1, and DQA1) has a role in risk (30). These approaches can be applied to non-HLA loci in the search for type 1 diabetes genes and, as an example, have been used in the study of DPT1 (ACE I/D polymorphism) and ACE level (31).

Perhaps one of the most carefully studied ethnically isolated populations (particularly with respect to the HLA region) is Sardinia (32-33). HLA-DR3 "marks" one of the two main type 1 diabetes susceptibility haplotypes in European and European-derived populations. However, its effect is heavily influenced by an unknown number of non-DR/DQ disease loci both within and outside the HLA region. In the Sardinian population the HLA-DQB1, -DQA1 and -DRB1 loci do not account for the entire association of HLA-DR3 (DRB1*0301-DQB1*0201) with type 1 diabetes risk (34-35). Using a stratification/conditional analysis approach, three regions outside DQ-DR but still within the MHC were considered to be "risk modifiers." The combined impact of these risk modifiers was substantial and highly significant (p = 6.2 x 10^{-7}). Haplotypes defined by SNPs within the DMB and DOB genes, and a TNF microsatellite locus identified 40% of Sardinian DR3+ haplotypes as non-predisposing (36).

This unique haplotype distribution and the high population frequency of DR3 found in Sardinia provide unique information that is needed to identify the sources of non-DR-DQ heterogeneity on DR3 haplotypes. Inclusion of selected diverse yet isolated populations (Sardinia, Finland, India and Africa) with the larger admixed populations (Caucasians from Europe, North America and Australia as well as United States African-Americans and Mexican-Americans), should provide important insights and facilitate type 1 diabetes gene identification.

2.   The INS Locus and IDDM2

In the early 1980's, a polymorphism in the 5' region of the insulin gene start site was observed (37-38). The resulting length polymorphisms were obvious candidates for their effect on susceptibility for either type 1 or type 2 diabetes. A series of early studies (36) confirmed an association of the *INS* "class I" allele of a variable number of tandem repeats (VNTR) polymorphism and type 1 diabetes, with an increase in the homozygote frequency in diabetics. More recent genetic and functional analyses in the region indicate that susceptibility to type 1 diabetes is likely to be directly influenced by the alleles of the *INS* VNTR (39). Genetic studies of allele and haplotype transmission in ~700 combined European and US families (40) revealed that the *INS*-VNTR-associated trait in type 1 diabetes was not recessive (with homozygosity of VNTR class I alleles providing risk). Rather, risk appeared to be largely a dominant trait with the VNTR class III alleles encoding protection from disease. While the biological mechanism underlying the genetic risk has yet to be resolved, variation defined by the VNTR may affect the steady-state level of insulin mRNA in the thymus, thereby influencing immune tolerance to insulin and its precursors. The precursors now represent the "favored" autoantigens in type 1 diabetes. Specifically, the class III VNTR alleles were associated with higher levels of *INS* mRNA in the thymus, which may account for its associated reduction of risk (or protection) for type 1 diabetes (41-44).

The role of *IDDM2/INS* and a possible interaction with HLA has yet to be fully resolved. Only one study has shown evidence of heterogeneity in type 1 diabetes risk encoded by the *INS* region according to HLA genotype (45). However, several other studies have reported that the association of the *INS* VNTR in different HLA risk classes (DR3/X, DR4/Y) is identical, suggesting that there is no evidence of heterogeneity by HLA and *INS* type. This lack of interaction between HLA and *INS* was recently confirmed using linkage in 356 affected sib-pair families from the UK (46).

3.   Other Genes and Regions

A number of studies have investigated associations and linkages with type 1 diabetes. While each site has been subject to ongoing investigation by single or multiple groups, there has been little coordinated effort to fully explore each region or their potential interactions. In part, this lack of effort is due to the recognition that the magnitude of non-HLA locus-specific effects is similar to that of *INS* (λs ~ 1.12) and others (λs < 1.3). In order to have sufficient statistical power, several thousand affected sib-pair families would be needed. Nonetheless, current regions of the genome in which linkage and/or association have been shown include 2q (*IDDM7-IDDM12, IDDM13*), 3 (*IDDM9*), 5q (*IDDM18*), 6 (*IDDM15, IDDM5*, and *IDDM8*), 10 (*IDDM10* and *IDDM17*), 11q (D11S1296-FGF3), and 14 (*IDDM14*). None of these loci, with the exception of association data for *CTLA4/IDDM12*, has yet to be confirmed, primarily due to the lack of sufficiently large samples of affected sib-pair families.

4.   Genetic Basis of Type 1 Diabetes in the Nonobese Diabetic (NOD) Mouse

The nonobese diabetic (NOD) mouse model is accepted as an accurate model of human type 1 diabetes (47-49). Most notably, the NOD mouse is sensitive to immunosuppression, both CD8 and CD4 T cells are a feature of its pancreatic histology, and insulin autoantibodies appear early in the disease process (50). Further, type 1 diabetes in human and mouse share a common dependence on the structure of peptide pocket P9 of the MHC class II orthologues HLA-DQ and IA. The NOD and its related strains have been used not only to examine the role of MHC genes, but also for detection of non-MHC susceptibility factors. In the mouse MHC (51), the common Kd and/or Db alleles of NOD mice contribute to the development of disease, but their contributions continue to be unclear. The MHC of the CTS/Shi mouse, originally designated as H2ct, shares the MHC class II region identity with the H2g7 haplotype of NOD mice. CTS mice, however, were reported to express different MHC class I gene products. Since the frequency of type 1 diabetes was reduced by over 50% in females of a NOD stock congenic for H2ct, the partial resistance could have been derived from the differences in the MHC class I genes. Recent evidence suggests that NOD/Lt and CTS/Shi share a common H2-Kd allele but differ at the H2-D-end of the MHC. The H2-D allele of CTS/Shi was identified as the rare H2-Ddx (described in ALR/Lt, another NOD-related strain). These data suggest that at least one protective MHC or MHC-linked genes in CTS mice may be at the H2-D end on the complex.

Owing to the special facility that mouse breeding offers, the dissection of complex traits, several type 1 diabetes susceptibility loci have been fine mapped to small chromosome regions. These loci include *Idd3* to 0.15 cM of proximal chromosome 3, *Idd10* and *Idd18*, also on chromosome 3 to a region less than 2 cM in size, *Idd5.1* on chromosome 1, *Idd13* on chromosome 2, and *Idd9.2* and *Idd9.3* on chromosome 4. All of these candidate regions (and more in the future) will be sequenced, revealing gene content and polymorphism (*e.g.,* NOD versus B6). For example, the mouse chromosome 1 *Idd5.1* interval contains *CTLA4*, the T cell regulatory gene (52). In addition, *IDDM12* is contained within the orthologous chromosome 2q33, a site that includes the *CTLA4* locus, which is the most strongly associated gene in the region when compared with flanking genes, *CD28* and *ICOS* (53). These experiments represent a portion of the work ongoing that provides an important source of candidate genes for human type 1 diabetes.

It was estimated that ~1,200 families may have genome scan data available and that data should be sent to the Coordinating Center for combined linkage analysis. The need to collect many more families was recognized, as was the desire to establish immortalized cell lines for a renewable source of DNA. Cell line availability was a key feature of the original UK and US collections. Trio (parents and type 1 diabetic child) and case-control collections were supported, particularly from distinct ethnic groups to facilitate trans-racial mapping approaches for etiological variant identification.

D.   SOURCES OF MATERIAL

Research material will be obtained in the form of blood samples and questionnaires regarding details of family history and treatment of diabetes and related conditions. The data will be collected for research purposes only.

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