Summary of the Type 1 Diabetes Genetics Consortium Autoantibody Workshop

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The Type 1 Diabetes Genetics Consortium (T1DGC) sponsored an Autoantibody Workshop, providing data from a large number of type 1 diabetes–affected sibling pair families with multiple autoantibodies assayed (both islet and nonislet targets) and extensive genetic and clinical information. Multiple groups analyzed the autoantibody data and various forms of genetic data. The groups presented their results at the T1DGC Autoantibody Workshop and compared results across genes and autoantibodies. The reports of the analyses of the autoantibody data with genetic information are contained as individual articles in this supplement. There were several consistent findings that emerged from the T1DGC Autoantibody Workshop. The human MHC (HLA genes) is the major contributor to variation in the presence of islet and nonislet autoantibodies in subjects with established type 1 diabetes. The contribution of non-MHC genes/variants to autoantibody prevalence is dependent on the set of single nucleotide polymorphisms tested, the autoantibody evaluated, and the inclusion criteria for sample selection. On the basis of these results, the HLA alleles *DRB1*0101* and *DRB1*0404* and the *PTPN22* R620W variant are consistently associated with autoimmunity in the T1DGC Autoantibody Workshop data.

Type 1 diabetes (T1D) results from the autoimmune destruction of the pancreatic β-cells, leading to absolute dependence on exogenous insulin to regulate blood glucose levels (1). T1D is a complex disease, with approximately one-half of the risk determined by genetic factors, and of that component, nearly one-half accounted for by the human MHC (HLA genes) (2). Following the first large-scale genome-wide association scan (GWAS) for T1D (3), conducted by the Type 1 Diabetes Genetics Consortium (T1DGC), the ImmunoChip, a custom high-density genotyping array, was designed and used to refine the mapping of T1D risk loci. The analysis of ImmunoChip data permitted the identification of the sets of most associated, or credible, single nucleotide polymorphisms (SNPs) in the risk loci. These analyses, together with prior GWAS findings, provide robust evidence for 44 T1D-associated genomic regions (4). Although significant disease-associated SNPs in these regions are located in/near genes, any assignment of causality to specific genes or variants should be treated with caution until enhancer-promoter interactions and other functional data are obtained.

With the recent large-scale GWAS and fine mapping in T1D, many risk loci and variants have been identified that differentiate case from control subjects. It should be noted, however, that the predictive value of these SNPs remains low, given the prevalence of T1D in the general population of ~0.5%. Thus, there remains a need to provide improved prediction of the development of T1D and better understanding of its natural history. It is generally accepted that T1D develops following an

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environmental trigger in a genetically susceptible host, resulting in an immune-mediated attack on the insulin-secreting β-cells of the pancreatic islets. This ongoing autoimmune attack is characterized by the appearance of one or more autoantibodies directed against islet-specific antigens. Increasing numbers of autoantibodies with different specificities are associated with a higher risk of progressing to clinical T1D. Recently, the TrialNet Natural History Study tested participants for the presence of autoantibodies to one of three antigens (GADA, IA-2A, and mIAA) at their initial screening (5). Any samples positive for at least one of these autoantibodies were then tested for two additional specificities, islet cell antibodies (ICA) and zinc transporter 8 (ZnT8) antibodies. An autoantibody risk score (ABRS) was developed that combined individual autoantibody levels along with their designations of positivity and negativity. The ABRS was strongly predictive of T1D, and receiver operating characteristic analysis of the ABRS revealed good predictability. The combination of the ABRS and the previously validated Diabetes Prevention Trial–Type 1 Risk Score (DPTRS) predicted T1D more accurately than either the DPTRS or the ABRS alone, confirming the importance of assessing the numbers of different autoantibodies present and the levels of those autoantibodies in predicting the risk of T1D.

Little is known about the influence of genetic variation on the presence of autoantibodies. Further, as there is known clustering of autoimmune diseases that is due, in part, to heritable factors (6), there is motivation for testing more than one set of organ-specific autoantibodies. The T1DGC was established to assemble collections of clinical data, DNA and biological materials, and genetic data and to conduct statistical genetic analyses on affected sibling pair families and unrelated case and control subjects to discover genes that modify risk of T1D. As part of this mission, the T1DGC distributed data and samples to bone fide investigators, conducted targeted scientific workshops, and supported training programs and technology dissemination. The T1DGC Autoantibody Workshop was one such effort with a goal of providing the T1DGC phenotypic, genotypic, and autoantibody data on affected sibling pair families to interested investigators for studies intended to discover genes accounting for variation in presence of autoantibodies. The T1DGC provided autoantibody and genetic data from 9,976 subjects from 2,321 affected sibling pair families to investigators taking part in the workshop. Seven groups analyzed the joint autoantibody and genetic data represented by six reports in this supplement, ranging from candidate gene analyses of selected autoantibodies to evaluation of regions of genetic variants associated with autoimmunity on the collection of autoantibodies.

The details of the workshop are provided in the individual articles in this supplement. The participants in the workshop operated in teams focused on specific (often nonoverlapping) questions; thus, rather than a competition of multiple methods on the same data set, the findings reflect the "consensus" of the working groups. The aim of this report is to summarize the findings of the different working groups as part of the T1DGC Autoantibody Workshop.

RESULTS FROM THE WORKSHOP

The assessment of autoantibodies focused on three islet autoantibodies as well as other organ-specific autoantibodies. It is known that the onset of T1D is preceded by the appearance of autoantibodies to a variety of islet cell antigens in serum, including insulin. In this workshop, insulin autoantibodies were not used for analysis. In genetically at-risk individuals, detection of multiple islet cell autoantibodies is a strong predictor for subsequent development of T1D. However, once T1D has become fully manifest, insulin autoantibody levels usually fall to low or undetectable levels, although, after insulin therapy is initiated, insulin autoantibody production may recur as a memory response. Thus, the determination of insulin autoantibodies in participants with T1D of varying duration as part of the T1DGC Autoantibody Workshop was not conducted.

Brorsson et al. (7) examined the association of 50 T1D-associated SNPs and HLA alleles with three islet autoantibodies (GADA, IA-2A, and ZnT8A) in 6,556 T1DGC subjects. In addition, nonislet autoantibodies (thyroid peroxidase [TPO], gastric parietal cells [PCA], transglutaminase [TG], and 21-hydroxylase [21-OH]) were tested for association with these T1D-associated SNPs and HLA. In addition to a strong association with HLA alleles, SNPs in five susceptibility loci (*IFIH1*, *PTPN22*, *SH2B3*, *BACH2,* and *CTLA4*) were significantly associated with more than one autoantibody at a false discovery rate (FDR) of less than 5%. The *IFIH1*/2q24 region exhibited a genome-wide significant association with PCA and was also associated, at a lesser significance level, with TPO autoantibodies (TPOA), GADA, and IA-2A. Eleven other loci were significantly associated (FDR $<$ 5%) with at least one autoantibody. In general, these T1D-associated loci that are also associated with autoantibodies across diseases appear to be involved in antigen presentation and T-cell receptor signaling and activation.

Wenzlau et al. (8) focused primarily on the role of HLA alleles and haplotypes associated with ZnT8A and other islet-specific autoantibodies. These analyses were performed on antibodies from sera collected within 3 years of T1D diagnosis, a time period when the ZnT8A are more likely to be present. ZnT8A were detected in 57.3% of subjects meeting this criterion, with the highest frequencies and median titers being associated with the shortest duration of disease. The C-terminal domain of *SLC30A8* (ZnT8) contains multiple autoantibody epitopes that can be separated into two classes depending upon residue 325. Class "A" epitopes have an absolute dependence on residue 325 and are directly related to the rs13266634 genotype. The sera from the T1DGC Autoantibody Workshop participants exhibited ZnT8A positivity in similar rates across multiple ethnic groups, and in non-Hispanic whites, ZnT8A positivity exhibited an association with the HLA-*DRB1*0401*-DQ8 haplotype. GADA showed a strong positive association with HLA-*DRB1*0301*-DQ2, while IA-2A showed an equally strong negative association with this haplotype. There was no significant association of HLA-*DRB1*0404*-DQ8 with single or dual autoantibody positivity.

Autoimmune thyroiditis occurs in 10–25% of patients with T1D, and the majority of subjects test positive for TPO antibodies. Previous studies of T1D alone and T1D combined with other autoimmune diseases implicated loci in the MHC (HLA-*DQB1* and HLA-*DRB1*) as

well as SNPs in two genes associated with T1D risk, *CTLA4* and *PTPN22*. Kahles et al. (9) analyzed T1DGC data by partitioning probands with T1D into two groups—those with TPOA (or thyroid disease) and those without TPO antibodies or thyroid disease. The focus of the genetic effects included the MHC (HLA loci) as well as *CTLA4*, *INS*, *PTPN22*, and *VDR*. In the T1DGC participants, the prevalence of TPO antibodies (25.2%) and thyroid disease (8.4%) was significantly associated with older age, female sex, and presence of other autoantibodies (GAD65, ATPase, 21-OH). The highest prevalence of TPO antibodies was in those of Hispanic ancestry (31%) and lowest in those of African ancestry (8%). In non-Hispanic whites, HLA-*DRB1*0101* was significantly less frequent in TPO-positive than in TPO-negative individuals; in contrast, HLA-*DRB1*0404*, HLA-*DQB1*0301*, and HLA-*DPB1*0201* were significantly more frequent in TPO-positive subjects. No significant differences were observed for association of TPO positivity or thyroid disease with SNPs in the *INS*, *CTLA4*, or *VDR* loci. There was a nominally significant ($P = 0.01$) association between TPO positivity and the *PTPN22* R620W variant (rs2476601).

Autoantibodies targeting the H+/K+-ATPase proton pump of the PCA are a highly specific and sensitive diagnostic of atrophic body gastritis (ABG) leading to pernicious anemia (PA). Wenzlau et al. (10) examined PCA, ABG, and PA in those with T1D and their relatives. PCA were detected in sera from 20.9% of 6,749 participants with T1D from the T1DGC and increased with age and were more prevalent in women (25.3%) than men (16.5%). PCA were more frequent among cases of T1D of Hispanic (36.3%) and African (26.2%) ancestry compared with non-Hispanic whites (20.8%) and Asian ancestry (16.7%). Estimates of heritability of PCA and other organ-specific autoantibodies (GAD65, IA-2, TPO, 21-OH, TG) ranged from 71% to 95%. PCA clustered with TPO, 21-OH, and persistent GAD65 autoantibodies, but not with celiac disease (TG) or IA-2A. PCA-positive subjects with T1D had an increased frequency of HLA-*DRB1*0404*, HLA-*DPB1*0201*, and *PTPN22* R620W (rs2476601) variants. PCA-positive subjects with T1D had a decreased frequency of HLA-*DRB1*0101*, HLA-*DPB1*0301*, and *CTLA4* CT60 (rs3087243) variants. Together, these variants accounted for a relatively small component (4–5%) of the heritable risk for PCA.

Autoimmune diseases cluster genetically, with alleles in some loci (e.g., the HLA genes in the human MHC and the R620W variant in *PTPN22*) contributing to the risk for multiple autoimmune diseases. Gutierrez-Achury et al. (11) contrasted the genetic background of T1D and celiac disease with respect to genetic susceptibility in order to explore the genetic differences between individuals developing both T1D and celiac disease (double autoimmunity) versus those with only one disease (T1D or celiac disease). A total of 42 SNPs associated with T1D and 28 SNPs associated with celiac disease were analyzed in 543 subjects with T1D who developed double autoimmunity. Comparison groups were composed of 2,472 T1D-only subjects and 2,223 celiac disease–only subjects. The *CTLA4* and *IL2RA* loci were more strongly associated with double autoimmunity than with either T1D or celiac disease alone. HLA-*DQ2.5*/*DQ8 (*where *DQ2.5* represents *DQA1*0501*, *DQB1*0201*, and *DRB1*03*, and *DQ8* represents *DQA1*03*, *DQB1*0302*, and *DRB1*04*), a T1D high-risk genotype (*DRB1*03*/*DRB1*04*), provided the greatest risk for developing double autoimmunity, consistent with a dominant role for HLA in multiple autoimmune processes.

CONCLUSIONS

The T1DGC Autoantibody Workshop provided multiple measures of organ-specific autoantibodies on a large series of genotyped families, ascertained for multiple cases of T1D. Seven groups of investigators worked on assembling and analyzing these data and presented findings at the workshop. Six reports of analyses are presented in this supplement. For details of the specific designs and antibodies and genes tested, please see each individual article in the supplement.

There were several consistent findings that emerged from the T1DGC Autoantibody Workshop. First, the human MHC (HLA genes) is the major contributor to variation in the presence of islet and nonislet autoantibodies in the context of T1D. The reported contribution of non-MHC loci to autoantibody prevalence is dependent, in part, on the SNPs tested (either global or selected) and the inclusion criteria used for sampling. Thus, when all samples were analyzed for all available genes, SNPs in *IFIH1*, *PTPN22*, *SH2B3*, *BACH2,* and *CTLA4* were associated with occurrence of multiple autoantibodies, while 11 SNPs were associated with single autoantibodies. In contrast, the focus of a specific autoantibody guided the selection of samples for analysis of ZnT8, as this autoantibody tends to be present only for a few years following diagnosis. Results in this smaller, selected set of participants with T1D identified ZnT8 positivity (and GAD65 and IA-2 positivity) as associated with HLA-*DRB1*0404*-DQ8. The MHC also played a role in TPO (or thyroid disease) positivity in participants with T1D. In this case, HLA-*DRB1*0101* was significantly less frequent (protective) in those TPO-positive subjects, while HLA-*DRB1*0404*, HLA-*DQB1*0301*, and HLA-*DPB1*0201* were significantly more frequent (risk) in TPO-positive subjects, with little effect of SNPs in the *INS*, *CTLA4,* or *VDR* genes (and nominal significance for *PTPN22*). PCA clustered with TPO, 21-OH, and GAD65 autoantibodies, but not with TG or IA-2 autoantibodies. PCA-positive subjects with T1D had an increased frequency of HLA-*DRB1*0404*, HLA-*DPB1*0201*, and the non-MHC *PTPN22* risk allele (R620W) and a decreased frequency of HLA-*DRB1*0101*, HLA-*DPB1*0301*, and the *CTLA4* CT60 SNP. Finally, in an evaluation of those subjects with T1D who also self-reported celiac disease, HLA-*DQ2.5*/*DQ8*, a T1D high-risk genotype, provided the greatest risk for developing double autoimmunity when compared with the two single-disease groups (T1D alone and celiac disease alone). Together, the HLA alleles HLA-*DRB1*0101* and HLA-*DRB1*0404* and the *PTPN22* R620W variant are consistently associated with autoimmunity in the workshop data.

An earlier report assessed the GADA, IA-2A, TPOA, and PCA autoantibody positivity on a genome-wide basis (12). GWAS SNPs were interrogated in subjects with T1D who had autoantibodies measured. Excluding the MHC locus, only two loci passed a stringent genome-wide significance level: 1q23/*FCRL3* (on the ImmunoChip) with IA-2A and 9q34/*ABO* (not on the ImmunoChip) with PCA. Eleven of 52 non-MHC T1D susceptibility loci showed evidence of association with at least one autoantibody. Associations

were largest in number with TPOA (*STAT4*, *BACH2*, *UBASH3A*, *PTPN22*, *CTLA4*, *IL2*, *RASGRP1*, *SH2B3*), followed by GADA (*IL2RA* and *SH2B3*), IA-2A (*IL27* and *IFIH1*), and PCA (*IFIH1*). Analysis of the TPOA-associated loci in 2,477 cases with Graves disease identified two novel autoimmune thyroid disease loci (*BACH2* and *UBASH3A*). The findings that *IFIH1* and *SH2B3* are associated with islet autoimmunity and *PTPN22* is associated with TPOA are consistent with the non-MHC loci identified in the T1DGC Autoantibody Workshop data.

Characterization of the natural history of T1D, starting from subjects at genetic risk and followed through the development of autoantibodies and overt clinical disease, is challenging. Part of this challenge is defining those at risk. Currently, there is increasing knowledge of the genes and variants that distinguish those subjects with T1D from those without, yet it is possible that the genetic factors that promote the autoimmune process (defined by islet autoimmunity) are different than, or overlap with, those that transition from islet autoimmunity to T1D. Longitudinal studies with frequent follow-up sampling are required to provide the framework to address this question. The Environmental Determinants of Diabetes in the Young (TEDDY) study has been established to identify environmental factors influencing the development of T1D using newborn screening and inclusion based upon T1D high-risk HLA alleles (13). TEDDY screened 414,714 infants, of which 19,906 (4.8%) were eligible, with ongoing follow-up and multiple ancillary projects focused on etiologic mechanisms.

TEDDY has evaluated the 41 non-HLA SNPs that achieved genome-wide significance for association with T1D in the GWAS meta-analysis conducted by the T1DGC (3). During the median follow-up time of 57 months in TEDDY, 350 children developed at least one persistent islet autoantibody (GADA, IA-2A, or mIAA) and 84 of them progressed to T1D. In 5,164 non-Hispanic white TEDDY participants (all having high-risk HLA genotypes), four SNPs were significantly associated with the development of islet autoimmunity and were located in or near *PTPN22*, *ERBB3*, *SH2B3*, and *INS* (14). While these studies are limited by the relatively low number of autoantibody-positive subjects and subjects who converted to T1D, continued follow-up should increase these sample sizes and include participants older than 9 years of age. Further, the process of screening included only those children with high-risk HLA genotypes, so the inference to the general population will be limited. Nonetheless, these approaches are required to delineate the impact of genetic variants on initiation and progression of islet autoimmunity and T1D.

In summary, the T1DGC Autoantibody Workshop has provided a novel resource for the examination of genetic factors that contribute to the variation in organ-specific immunity in the context of T1D. A variety of analytic approaches and studies of subsets of participants and genetic variants revealed that there are several consistent findings. The HLA alleles *DRB1*0101* and *DRB1*0404* and the *PTPN22* R620W variant are consistently associated with autoimmunity in the T1DGC Autoantibody Workshop data. While many combinations of genetic factors and subgroup analyses remain for future examination, these results add to our knowledge of autoantibody variation in T1D.

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