Design and Measurement of Nonislet-Specific Autoantibodies for the Type 1 Diabetes Genetics Consortium Autoantibody Workshop

Beena Akolkar,[1] Joan Hilner,[2] and Concepcion R. Nierras[3]

The Type 1 Diabetes Genetics Consortium (T1DGC) comprised groups of investigators from many countries throughout the world, with a common goal of identifying genes predisposing to type 1 diabetes. The T1DGC ascertained and collected samples from families with two or more affected siblings with type 1 diabetes and generated a broad array of clinical, genetic, and immunologic data. The T1DGC Autoantibody Workshop was designed to distribute data for analyses to discover genes associated with autoantibodies in those with type 1 diabetes. In the T1DGC-affected sibling pair families, three T1DGC Network laboratories measured antibodies to the islet autoantigens GAD65 and the intracellular portion of protein tyrosine phosphatase (IA-2A). The availability of extensive genetic data provided an opportunity to investigate the associations between type 1 diabetes and other autoimmune diseases for which autoantibodies could be measured. Measurements of additional nonislet autoantibodies, including thyroid peroxidase, tissue transglutaminase, 21-hydroxylase, and the potassium/hydrogen ion transporter H+/K+-ATPase, were performed by the T1DGC laboratory at the Barbara Davis Center for Childhood Diabetes, Aurora, CO. Measurements of all autoantibodies were transmitted to the T1DGC Coordinating Center, and the data were made available to members of the T1DGC Autoantibody Working Groups for analysis in conjunction with existing T1DGC genetic data. This article describes the design of the T1DGC Autoantibody Workshop and the quality-control procedures to maintain and monitor the performance of each laboratory and provides the quality-control results for the nonislet autoantibody measurements.

[1]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD
[2]Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL
[3]JDRF, New York, NY

Corresponding author: Concepcion R. Nierras, concepcion.nierras@nih.gov.

The international Type 1 Diabetes Genetics Consortium (T1DGC) comprised groups of investigators from many countries throughout the world, with a common goal of identifying genes predisposing to type 1 diabetes. The T1DGC assembled a collection of ~4,000 type 1 diabetes–affected sibling pair (ASP) families, as well as a large series of case-control collections for genetic studies. The T1DGC organized four recruitment networks (Asia-Pacific, Europe, North America, and U.K.) for collection of data and samples (1,2), and, within these networks, laboratories were chosen for processing biological samples (3). Three laboratories were selected to serve the T1DGC, initially to measure islet autoantigens from the baseline samples (4). These autoantigens were GAD65 and the intracellular portion of protein tyrosine phosphatase (IA-2A).

The T1DGC developed a masked split-pair duplicate sample program that allowed assessment of intra- and interassay reproducibility over time for each of the assays.

These assessments included evaluation of different methods of computing results reported in World Health Organization (WHO) units per milliliter for sera yielding signals above the highest WHO standard. The results of the Diabetes Autoantibody Standardization Program (DASP) for the three laboratories have previously been described (4) for islet autoantibodies. The DASP workshops have provided insights for the improvement and standardization of autoantibody measurement associated with type 1 diabetes across numerous laboratories, and performance in DASP was used as a criterion for selecting the laboratories and for monitoring their performance.

Autoantibodies against GAD and IA-2A were measured in samples from participants with type 1 diabetes from T1DGC ASP families. Although the measurement was not used as an entry criterion for participation in the study, quantifying results in standardized WHO units per milliliter allowed more detailed phenotyping and increased the research value of T1DGC study samples.

With recognition of the clustering of multiple autoimmune diseases and overlap among genes contributing to multiple autoimmune diseases, additional organ-specific antibodies were measured in T1DGC samples. These additional assays were for antibodies to thyroid peroxidase (TPO) for Hashimoto disease, to transglutaminase (TG) for celiac disease, to 21-hydroxylase (21-OH) for Addison disease, and to H+/K+-ATPase (ATPase) for pernicious anemia (5).

The T1DGC Autoantibody Workshop was designed to distribute data for analyses to discover genes associated with autoantibodies in participants with type 1 diabetes. Results of these analyses are reported in other articles in this supplement. This article describes the design of the T1DGC Autoantibody Workshop and the quality-control (QC) procedures to maintain and monitor the performance of each laboratory with respect to nonislet autoantibodies.

RESEARCH DESIGN AND METHODS

The design of the T1DGC Autoantibody Workshop consisted of the T1DGC making data available to analytic teams to apply methods to detect genetic variants that contribute to the variation observed in autoantibody measurements. The primary data sources are 1) the clinical phenotypic data associated with the collected samples from participants with type 1 diabetes, 2) the genetic data from multiple T1DGC experiments, 3) previously generated islet autoantibody measurements, and 4) newly generated nonislet autoantibody data. An announcement was made to the research community of the T1DGC Autoantibody Workshop and availability of data. Cleaned data sets were made available to the research teams (investigators interested in the same topic were encouraged to form "teams") and results of the analyses presented at an in-person meeting. Articles representing the results of these analyses are found in this supplement.

Each of the first three sources of data (clinical phenotypes, genotypes, and existing islet autoantibodies) had undergone extensive QC procedures. Details of the generation, QC, and analyses of the assembled genetic data can be found in previous reports (6–10). The methods for the autoantibody assays are described in greater detail in the articles in this supplement as well as in other summaries (4,5). The previously measured islet autoantibodies were assayed in the three T1DGC laboratories.

Measurements of the organ-specific autoantibodies (TPO, TG, 21-OH, and ATPase) were performed in the T1DGC North American laboratory (Barbara Davis Center for Childhood Diabetes, Aurora, CO), which was designated as the Autoantibody Workshop Laboratory. This article describes the QC components for the nonislet autoantibody measurements used in the T1DGC Autoantibody Workshop.

Autoantibody QC Procedures

The details on tracking assay performance for the measurement of islet autoantibodies have previously been reported (4). Assay results were reported to the T1DGC Coordinating Center.

Measurements of nonislet autoantibodies were performed at a single T1DGC Autoantibody Workshop Laboratory at the Barbara Davis Center. Similar to the QC assessment procedure for islet autoantibodies, a two-pronged QC system was implemented to assess nonislet autoantibodies. First, univariate analyses were conducted by the Coordinating Center (Wake Forest University) on the monthly data results from the T1DGC Autoantibody Workshop Laboratory. Briefly, results over time were recorded and evaluated for drift. Based on these analyses, summary statistics (e.g., means, variances) and out-of-range values were obtained and, if necessary, investigated further. Second, duplicate autoantibody measures were performed on a random sample of ~5% of participants with type 1 diabetes. Duplicate sera were collected and labeled with a separate, unique identifier and were sent to the laboratory in the normal sample shipments. The laboratory staff were masked as to which samples were duplicates. These samples were often measured in the same assay and are representative of intra-assay variation.

Interassay variation was evaluated by a second split-duplicate protocol in which previously measured duplicate pairs were resubmitted to the laboratory. A total of 632 intra-assay split pairs were tested for each autoantibody evaluated in the T1DGC Autoantibody Workshop. Reliability was assessed using intraclass correlations and the technical error measurement for autoantibody measures, in addition to graphical inspection of the data. The technical error is defined as the square root of the pooled between-measures variance as a percentage of the sample mean. The technical error was compared with the laboratory's internal coefficient of variation (CV). If there was evidence of high technical error, the laboratory was contacted and the cause determined. The results of split-duplicate determinations were expressed as antibody positive or negative (as defined within the laboratory) and as WHO units per milliliter or as indices, depending on the autoantibody. For the Autoantibody Workshop, autoantibody measurements were certified and provided by the T1DGC Coordinating Center.

Genetics Data and QC Procedures

The available genetic data in the MHC on chromosome 6p21.3 were derived from the T1DGC MHC Fine Mapping project (11). These genetic data consisted of a dense single nucleotide polymorphism (SNP) map ($2 \times 1,536$ SNP panels), a polymorphic microsatellite map (66 variable number tandem repeats), and classic HLA typing to ensure comprehensive coverage of the 4-Mb MHC core region (Ensembl positions 29.5–33.5 Mb). Specific QC procedures were developed and implemented for each genotyping protocol (11). Quality checks, including examination of plate-to-panel yields,

SNP performance, Hardy-Weinberg equilibrium expectations, mismatch error rates, Mendelian error rates (within families), and allele distribution patterns, were conducted for all data. Similar QC methods were implemented for candidate genes (T1DGC Rapid Response Workshop) (12), genome-wide linkage analyses (6–8), and the ImmunoChip analysis (conducted at the University of Virginia [10]).

Samples

The DNA samples used for these studies were derived from T1DGC ASP families, representing a total of 9,992 individuals from 2,325 ASP families ascertained from nine T1DGC recruitment cohorts (11). The families consisted primarily of nuclear families with an ASP, often with two parents and an unaffected sibling. After exclusions for poor-quality DNA, there were 9,979 samples and 339 QC samples sent for genotyping for all genetic marker sets.

RESULTS

The data based on the islet autoimmune markers used in the T1DGC Autoantibody Workshop are provided elsewhere (4,10,11).

For the Autoantibody Workshop, T1DGC recognized the opportunity to investigate genetic associations between type 1 diabetes and other autoimmune diseases for which autoantibodies could be measured. Nonislet autoantibodies were measured in samples from participants with type 1 diabetes; the nonislet antibodies are focused on the thyroid, adrenal, and parietal cell (gastric) systems. The results of QC that produced the analytic data for nonislet antibody intra-assay reproducibility are provided here. A summary of the QC results for these autoantibodies is provided in Table 1.

TPO is an enzyme found in the thyroid gland that contributes to the production of thyroid hormones. The presence of TPO antibodies suggests the presence or development of autoimmune thyroid disease (Hashimoto thyroiditis or Graves disease). All samples were tested for TPO antibodies in the T1DGC Autoantibody Workshop Laboratory. The technical error for samples was 7.74%. The internal CV at low TPO levels was 6.1% and at high levels was 8.7%. A total of 632 intra-assay split pairs were used for TPO testing. The mean (SD) difference between the pairs was 0.82 (10.92) WHO units/mL with 95.9% concordance in positive/negative calls within the split-pair samples.

TG antibodies bind TG, a major thyroid-specific protein that is important in thyroid hormone synthesis, storage, and release. TG antibodies and TPO antibodies are used in concert to identify likely cases of autoimmune thyroiditis (Hashimoto and Graves disease). The values for this autoantibody are expressed as an index. The technical error for samples was 0.01%, and the internal CV was 5.9%. A total of 632 intra-assay split pairs have been tested for TG. The mean (SD) difference between the pairs was 0.00 (0.02) with 98.7% concordance in positive/negative calls within the split-pair samples.

The microsomal 21-OH (55 kD) has been shown to be the primary autoantigen associated with autoimmune Addison disease. The 21-OH autoantibodies are markers of autoimmune destruction of the adrenal cortex. In the T1DGC Autoantibody Workshop, the values are expressed as an index, with a technical error for samples of 0.01%. The internal CV at low 21-OH autoantigen levels was 6.1%, and the internal CV at high levels was 8.7%. A total of 632 intra-assay split-pair samples were tested for 21-OH. The mean (SD) difference between the pairs was 0.00 (0.02) with 100% concordance in positive/negative calls within the split pairs.

Pernicious anemia is characterized by atrophic body gastritis leading to autoimmune chronic atrophic gastritis with destruction of gastric parietal cells and an inability to absorb vitamin B_{12}. Diagnosis of pernicious anemia includes the presence of parietal cell antibodies that bind to the ATPase. For ATPase autoantibodies in the T1DGC samples, the technical error was 0.02%. Every plate of samples in the assay had one well derived from a pool of samples that historically had the very high titers as well as a series of five historically low titer samples as negative controls. The mean of intra-assay CVs was used to represent the typical CV for this assay: 2.6% (high titers), 11.1% (controls), and 4.5% (midrange values). A total of 632 intra-assay split pairs have been tested for ATPase. The mean (SD) difference between the pairs was 0.00 (0.03) with 97.9% concordance in positive/negative calls within the split pairs.

CONCLUSIONS

The purpose of the T1DGC Autoantibody Workshop was to provide data sets for investigators to be able to determine the contribution of genes to variation in the presence of autoantibodies in a large collection of families enriched for type 1 diabetes (T1DGC ASP families). The T1DGC ASP families had previously been characterized with islet autoantibodies. Given the co-occurrence of other organ-specific autoimmune diseases with type 1 diabetes, there was interest in determining the presence of nonislet autoantibodies in family members with type 1 diabetes. With the extensive genetic data for analysis, a critical component of the workshop was to provide the antibody phenotypes to the working groups with robust measurements and QC.

Measurements were made of nonislet autoantibodies focused on the thyroid (TPO and TG), adrenal (21-OH), and parietal cell (gastric ATPase) systems in T1DGC participants with type 1 diabetes. We evaluated intra-assay precision, using masked sera, with a split-duplicate sample program. With more than 632 masked duplicate samples sent directly from the clinics and mixed with other samples, we determined the intra-assay percentage concordance of original positive/negative calls for selected autoantibodies. For the assays used in the T1DGC Autoantibody Workshop, there was high concordance in positive/negative

Table 1—QC split-pair results for TPO, TG, 21-OH, and ATPase autoantibodies

	TPO	TG	21-OH	ATPase
Mean difference	0.82	0.00	0.00	0.00
SD	10.92	0.02	0.02	0.03
Percent concordance	95.9	98.7	100.0	97.9
R	0.98	0.98	0.98	1.00
Technical error	7.74	0.01	0.01	0.02
Internal CV	6.1, 8.7	5.9	6.1, 8.7	4.5, 2.6

calls within the duplicate split pairs, ensuring high-quality data for the genetic analyses of the samples.

References

1. Hilner JE, Perdue LH, Sides EG, et al.; T1DGC. Designing and implementing sample and data collection for an international genetics study: the Type 1 Diabetes Genetics Consortium (T1DGC). Clin Trials 2010;7(Suppl.):S5–S32
2. Rosinger S, Nutland S, Mickelson E, et al.; T1DGC. Collection and processing of whole blood for transformation of peripheral blood mononuclear cells and extraction of DNA: the Type 1 Diabetes Genetics Consortium. Clin Trials 2010;7(Suppl.):S65–S74
3. Perdue LH, Albret L, Aldrich A, et al.; T1DGC. Quality control of phenotypic forms data in the Type 1 Diabetes Genetics Consortium. Clin Trials 2010;7(Suppl.):S46–S55
4. Bingley PJ, Williams AJ, Colman PG, et al.; T1DGC. Measurement of islet cell antibodies in the Type 1 Diabetes Genetics Consortium: efforts to harmonize procedures among the laboratories. Clin Trials 2010;7(Suppl.):S56–S64
5. Wenzlau JM, Hutton JC. Novel diabetes autoantibodies and prediction of type 1 diabetes. Curr Diab Rep 2013;13:608–615
6. Concannon P, Erlich HA, Julier C, et al.; Type 1 Diabetes Genetics Consortium. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. Diabetes 2005;54:2995–3001
7. Concannon P, Chen WM, Julier C, et al.; Type 1 Diabetes Genetics Consortium. Genome-wide scan for linkage to type 1 diabetes in 2,496 multiplex families from the Type 1 Diabetes Genetics Consortium. Diabetes 2009;58:1018–1022
8. Morahan G, Mehta M, James I, et al.; Type 1 Diabetes Genetics Consortium. Tests for genetic interactions in type 1 diabetes: linkage and stratification analyses of 4,422 affected sib-pairs. Diabetes 2011;60:1030–1040
9. Barrett JC, Clayton DG, Concannon P, et al.; Type 1 Diabetes Genetics Consortium. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. Nat Genet 2009;41:703–707
10. Onengut-Gumuscu S, Chen WM, Burren O, et al. Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. Nat Genet 2015;47:381–386
11. Brown WM, Pierce J, Hilner JE, et al.; Type 1 Diabetes Genetics Consortium. Overview of the MHC fine mapping data. Diabetes Obes Metab 2009;11(Suppl. 1):2–7
12. Brown WM, Pierce JJ, Hilner JE, et al.; Type I Diabetes Genetics Consortium. Overview of the Rapid Response data. Genes Immun 2009; 10(Suppl. 1):S5–S15