Appendix A

GoKinD Methods

Processing of samples at the clinical centers. Informed consent was obtained from participants in the United States and Canada. Samples for analyses and for provision of DNA (both transformed cells and extracted DNA) were collected and processed. The samples for extraction of DNA and the blood for isolation of peripheral blood mononuclear cells (PBMCs) were sent promptly to the CBL at the University of Minnesota by overnight courier. Collected under non-fasting conditions, serum, plasma, and urine samples were frozen and stored locally and were sent in batches to the CBL. Samples for HbA_{1c} were refrigerated and sent in batches to the CBL within 5 days of collection.

Isolation, processing and storage of transformed PBMCs. Upon receipt (same or next day), PBMCs were promptly isolated by centrifugation with a Ficoll gradient, diluted in fetal calf serum and dimethylsulfoxide (DMSO), and frozen in the vapor phase of liquid nitrogen. Later the PBMCs were thawed, transformed with Ebstein-Barr virus (EBV), and placed into cell culture in enriched RPMI-1640 media. After sufficient time for adequate growth of the cells (ranging from weeks to 2 - 3 months), aliquots of the proliferating cells were taken and frozen in the vapor phase of liquid nitrogen for future provision of DNA through subsequent growth of the transformed cells. To date, the transformation success rate in GoKinD samples has been 2354 of 2360 or 99.8%.

Assessment of glycemic control, renal function, and lipids. HbA_{1c} was determined by ion-exchange high performance liquid chromatography (HPLC) using a Tosoh A1c 2.2 Plus (Tosoh Medics, Inc., Foster City, CA) at the CBL. Non-fasting serum creatinine was measured by a variation on the Jaffe method, and cystatin-C was measured by an immunoassay using antibody-coated latex particles on the Dade Behring BN 100 nephelometer (Dade Behring Inc., Deerfield, IL). Serum cholesterol (by an enzymatic procedure) and HDL-cholesterol (by the measurement of cholesterol after magnesium dextran precipitation) were assayed in a laboratory certified by NHLBI-CDC and referenced to Diabetes Control and Complications Trial (DCCT) values. Random urine collections were obtained for the determination of albuminuria, with creatinine measured by a variation on the Jaffe method, and albumin in urine measured by a fluoroimmunoassay in the laboratory of Blanche Chavers (DCCT references). The results for the albumin creatinine ratio (ACR) were expressed in µg albumin/mg creatinine.

QC for clinical measurements. In GoKinD, replicate samples from 5% of the participants were collected to serve as quality control materials. In this multi-center study with a central laboratory, the specimens were collected locally, processed, aliquoted, labeled, shipped, received at the central laboratory, processed, and assayed, and the results were reported back to the clinical center. Because any one of these steps could introduce errors in the observed measurements, all aspects of sample processing and data reporting were included in the QC process. The best way to assess the reliability of the total

system, therefore, is to duplicate all of the steps when evaluating within specimen variation (1). The coefficient of reliability, the proportion of total variation observed between specimen values due to differences in the true values, and the 95% confidence interval are the best measures of reproducibility. One minus the reliability coefficient is the proportion of variation among observed values that is due to error. Table 3 presents the coefficients of reliability and the 95% confidence intervals for the 7 clinical measurements and the calculated albumin/creatinine ratio in GoKinD.

DNA isolation and quantification. A 10 mL EDTA whole blood tube was collected for DNA isolation from each participant. The CBL received all EDTA blood tubes within 24 hours of the blood draw and began the DNA isolation process using the commercially available Puregene DNA Isolation kit (Gentra Systems, Inc., Minneapolis, MN). When the cell lysis solution is added to the processed sample, the lysate is stable for at least 18 months at room temperature (Gentra Systems, Inc., Minneapolis, MN). The CBL then shipped the processed lysate to CDC where the isolation process was completed according to the manufacturer's instructions. To solubilize the DNA pellet, the sample was incubated at 65^0C for 4 hours. The sample was then rocked on an orbital rocker for 7 days at room temperature to ensure the sample was completely in solution. To determine the DNA concentration of the sample, three 1:20 dilutions were evaluated at A_{260} and A_{280} using the Spectromax spectrophotometer (Molecular Devices, Sunnyvale, CA). For each dilution, the A_{260} and A_{280} were repeated 5 times to ensure accuracy.

Genotyping Methods. All HLA genotyping methods were based on polymerase chain reaction (PCR) amplification from genomic DNA of the exon to be evaluated, followed by florescent-based cycle sequencing on the ABI 3100 or ABI 3730 (Applied Biosystems, Foster City, CA). The sequence data were then analyzed using the Applied Biosystems MatchTools analysis package (Foster City, CA) and, recently, using the Conexio Genomics Assign program (Western Australia). The DRB1 genotyping method evaluated the identity of polymorphisms located in exon 2 using the commercially available AlleleSEQR DRB1 kit (Atria Genetics, San Francisco, CA). The HLA DQA1 genotyping method, which was developed at CDC (2, 3), evaluated the identity of the polymorphisms located in exons 2 and 3 for all participants. In the case of the *0302 and *0303, and the *010101, *010102, *010401, and *0105 ambiguities, exon 1 was also evaluated in appropriate participants to further resolve these ambiguous genotypes. The HLA DQB1 genotyping method evaluates the identity of polymorphisms located in exons 2 and 3 using a method also developed at CDC.

The genotyping method for the –23 insulin gene SNP involved a standard allelic discrimination reporter assay using florescent labeled reporter probes. These genotype assignments are confirmed with real time PCR on the ABI 7900 (Applied Biosystems, Foster City, CA).

Microsatellites (TH01, TPOX, CSF1PO) and Amelogenin (a sex specific locus) were evaluated for each participant for quality control. The three microsatellites, TH01, TPOX, and CSF1PO and Amelogenin loci were co-amplified with florescent labeled primers using a commercially available AmpFLSTR Green I PCR Amplification kit

(Applied Biosystems, Foster City, CA). The number of repeats that an individual has at each locus was determined using the ABI 3100 Fragment Analyzer with the Genemapper ID software version 3.1 (Applied Biosystems, Foster City, CA). In the event that a sample was determined problematic, nine additional microsatellites were evaluated using the commercially available Profiler Plus kit (Applied Biosystems, Foster City, CA). The microsatellites included in this assay were D3S1358, vWA, D18S51, FGA, D5S818, D8S1179, D13S317, D21S11, and D7S820.

Genetic QC at CDC. All assays were run with a positive DR3/DR4 control and a negative control. The positive control checked for the integrity of the assay (such as amplification of both DR3 and DR4 alleles, machine function, and genotyping analysis). The negative control checked for contamination of reagents. Additionally, all samples were genotyped for three polymorphic microsatellites (TH01, TPOX, and CSF1PO) known to be useful in the forensic community as well as for the Amelogenin sex marker. The sex marker verified the individual's sex, and the three microsatellites, along with the polymorphic HLA genotypes, detected sample mix-ups and verified biological relationships. If a problem was detected, the assays in question were repeated, and the family was then genotyped for 9 additional microsatellites (D3S1358, vWA, D18S51, FGA, D5S818, D8S1179, D13S317, D21S11, D7S820). Because these microsatellite tests are extremely sensitive, this assay also allows for the detection of even slight sample contamination. CDC also checked for internal laboratory sample mix-ups or contamination of samples, by performing a blinded repeat of 5% of all samples received. When additional genomic DNA was required, CDC received transformed lymphocyte lysate from the CBL for DNA extraction. The identity of all duplicate samples was verified against the original blood sample using the above three microsatellites (TH01, TPOX and CSF1PO) and the Amelogenin sex marker.

Data QC at CDC. All analyzed data entered into the database were independently checked against the raw data for entry errors. The HLA haplotypes were compared with known haplotypes found in previous studies. The haplotypes for trios were determined by looking at parental transmission, and haplotypes for singletons were estimated based on known haplotypes. If the haplotype was unusual, alleles were checked against ethnic-specific data and the assays were repeated if necessary.

References

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