PROTOCOL

The Genetics of Kidneys in Diabetes (GoKinD) Study

Proposed implementation

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List of Acronyms

ACE	Angiotensin-Converting Enzyme
ACR	Urine Albumin/Urine Creatinine Ratio
ARC	Application Review Committee
BDA	British Diabetic Association (now Diabetes UK)
CBL	Central Biochemistry Laboratory
CDC	Centers for Disease Control and Prevention
CHS	Committee on Human Studies
CPT	Cell Preparation Tube
Diabetes UK	Diabetes United Kingdom (formerly British Diabetic Association)
DNA	Deoxyribonucleic Acid
EDIC	Epidemiology of Diabetes Interventions and Complications
EDTA	Ethylenediaminetetraacetic Acid
ESRD	End-Stage Renal Disease
FedEx	Federal Express
FSGS	Focal Segmental Glomerulosclerosis
FTP	File Transfer Protocol
GoKinD	Genetics of Kidneys in Diabetes
GWU (or GW)	The George Washington University
HbA1c	Hemoglobin A1c
HIV	Human Immunodeficiency Virus
HLA	Human Lymphocyte Antigen
HTML	Hypertext Machine Language
IDDM	Insulin-Dependent Diabetes Mellitus
IgA	Immunoglobulin A
IgM	Immunoglobulin M
IRB	Institutional Review Board
JDRF	Juvenile Diabetes Research Foundation
MI	Myocardial Infarction
MMG	Matthews Media Group
MPA	Multiple Project Assurance
NCBI	National Center for Biotechnology Information
NIDDK	National Institute of Diabetes & Digestive & Kidney Diseases
NIH	National Institutes of Health

OHRP	Office for Human Research Protections
POC	Publications Oversight Committee
RAB	Research Advisory Board
RFLP	Restriction Fragment Length Polymorphism
SHC	Specimen Handling Committee
SNP	Single Nucleotide Polymorphism
SPA	Single Project Assurance
U.S.A.	United States of America

A. Introduction and Specific Aims

Epidemiologic and family data provide strong evidence for the existence of genetic susceptibility to diabetic nephropathy. Recent developments in molecular genetics have made it possible to search for the genes responsible for this susceptibility. At present, the major factor limiting progress in this effort is the availability of sufficient DNA samples from families with and without diabetic nephropathy that have been carefully phenotyped.

The overall goal of the GoKinD ("Genetics of Kidneys in Diabetes") study is to establish a repository of DNA and clinical information from a large number of unrelated patients with type 1 diabetes in order to facilitate studies into the genetic basis of diabetic nephropathy. The specific goals are to:

1) Ascertain, recruit and examine 600 type 1 diabetic patients with clinically diagnosed diabetic nephropathy together with their parents.
2) Ascertain, recruit and examine 500 type 1 diabetic patients with clinically diagnosed diabetic nephropathy for whom parents are not available.
3) Ascertain, recruit and examine 500 type 1 diabetic patients with normoalbuminuria and diabetes duration at least 15 years together with their parents.
4) Ascertain, recruit and examine 500 type 1 diabetic patients with normoalbuminuria and diabetes duration at least 15 years for whom parents are not available.
5) Characterize endpoints related to diabetic nephropathy and collect baseline information on other complications through examination and medical record review.
6) Extract DNA from blood samples for immediate use (at least 100 μg DNA) and establish lymphoblast cell lines as a future source of DNA.
7) Maintain an inventory of the DNA samples, clinical data, and genotype data for individuals included in the JDRF collection.
8) Develop databases to foster compatibility with a similar resource being assembled in the United Kingdom under the sponsorship of the British Diabetic Association (BDA).

The fundamental aim of GoKinD is to facilitate investigator-driven research into the genetic basis of diabetic nephropathy. Decisions regarding the genes and chromosomal regions to be studied will be made by individual investigators and subject to a competitive review process. This design will ensure the best use of the resource to elucidate the role of genetic variants in diabetic nephropathy.

The following two tables summarize power of affected child trios (ACTs) and unaffected child trios (UCTs) to detect transmission distortion. The number of trios being collected for GoKinD is sufficient to detect association for many, but certainly not all situations. However, three points should be made. First, our design includes both ACTs and UCTs, giving substantially greater coverage in terms of the number of models for which transmission distortion will be detected. Second, ACTs and UCTs can be analyzed together, increasing the power considerably in comparison to using either design alone. Finally, a similar data set is also being collected in the UK, effectively doubling sample sizes.

Table I. Ratios of sample sizes (UCTs/ACTs) required to achieve 80% power to detect transmission distortion of a 50% frequent risk allele

Model	Baseline Penetrance	Risk Ratio = 1.5				Risk Ratio = 2.0			
		Prevalence	ACT Sample Size	UCT Sample Size	Ratio	Prevalence	ACT Sample Size	UCT Sample Size	Ratio
Dominant	0.2	0.28	934	6632	7.1	0.35	373	1339	3.6
	0.3	0.41	934	1942	2.1	0.53	373	320	**0.86**
	0.4	0.55	934	644	**0.69**	0.70	373	71	**0.19**
Recessive	0.2	0.23	644	7504	11.7	0.25	199	1745	8.8
	0.3	0.34	644	2426	3.8	0.38	199	532	2.7
	0.4	0.45	644	934	1.5	0.50	199	187	**0.93**
Additive	0.2	0.30	280	1536	5.5	0.40	123	280	2.3
	0.3	0.45	280	419	1.5	0.60	123	53	**0.43**
	0.4	0.60	280	123	**0.44**	-	-	-	-
Multiplicative	0.2	0.31	195	944	4.8	0.45	69	101	1.5
	0.3	0.47	195	248	1.3	-	-	-	-
	0.4	0.62	195	67	**0.34**	-	-	-	-

Note. – Models that are not legal are denoted by a "-"

Table II. Comparison of relative efficiencies for unexposed (Un), exposed (Ex), and total groups of ACTs and UCTs for four types of interaction assuming that the genetic risk is the same for one or two alleles (i.e., $\psi_1 = \psi_2$)*.

Interaction	Baseline Penetrance#	Risk ratio ($\psi_1=\psi_2$)			Disease prevalence			Required size	Relative efficiency vs. total ACTs				
		Un	Ex	Total	Un	Ex	Total	Total ACTs	Total UCT	Un ACT	Un UCT	Ex ACT	Ex UCT
Synergistic	0.1	1.5	2.25	1.73	0.12	0.25	0.16	495	28.6	1.9	104	**0.59**	5.4
	0.2	1.5	2.25	1.73	0.24	0.51	0.32	495	4.7	1.9	19.5	**0.59**	**0.59**
	0.3	1.5	2.25	1.73	0.36	0.76	0.48	495	1.23	1.9	6.17	0.59	**0.061**
	0.4	1.5	2.25	1.73	-	-	-	-	-	-	-	-	-
None	0.1	1.5	1.5	1.5	0.12	0.18	0.14	**934**	40.1	**1**	55.2	**1**	21.4
	0.2	1.5	1.5	1.5	0.24	0.36	0.28	**934**	7.1	**1**	10.3	**1**	3.3
	0.3	1.5	1.5	1.5	0.36	0.54	0.41	934	2.1	1	3.3	1	**0.76**
	0.4	1.5	1.5	1.5	0.48	0.72	0.55	934	0.69	1	1.2	1	**0.16**
Sub-multiplicative	0.1	1.5	1.33	1.45	0.12	0.16	0.13	1115	43.5	**0.81**	44.8	1.5	40.5
	0.2	1.5	1.33	1.45	0.25	0.33	0.27	1115	7.8	**0.81**	8.4	1.5	6.6
	0.3	1.5	1.33	1.45	0.36	0.49	0.40	1115	2.3	**0.81**	2.7	1.5	1.7
	0.4	1.5	1.33	1.45	0.48	0.65	0.53	1115	0.80	0.81	1.0	1.5	**0.44**
Antagonistic	0.1	1.5	1	1.35	0.12	0.13	0.12	2023	49.4	**0.46**	25.5	-	-
	0.2	1.5	1	1.35	0.24	0.26	0.25	2023	9.6	**0.46**	4.8	-	-
	0.3	1.5	1	1.35	0.36	0.39	0.37	2023	3.0	**0.46**	1.5	-	-
	0.4	1.5	1	1.35	0.48	0.52	0.49	2023	1.1	**0.46**	0.56	-	-

Note. – Models that are not legal and non-defined quantities are denoted by a "-". The best trio type is indicated by boldface type.

UCT sample size can be determined by multiplying ACT sample size by relative efficiency.

* The risk allele frequency was 50%. 30% of the total group is exposed.

Baseline penetrance for the total group.

Power (80% at the 5% level) for the case-control design is as follows. If allele frequency is .1 in cases and .2 in controls (or vice versa), 199 case alleles and 199 control alleles would be sufficient to detect association. For frequencies of .2 and .3, 294 of each is required. For .3 and .4, 356 of each is required. For .4 and .5, 388 of each is required. Other situations of allele frequency differences of .1 (i.e., when both frequencies are >50%) are exactly the same owing to the symmetric nature of the binomial distribution. This implies that our sample of 500 cases and 500 controls (which produces 1000 case alleles and 1000 control alleles) is sufficiently large to detect at least differences of .1 in allele frequency. If many cases, we will be able to detect much smaller differences. For example, 453 cases and 453 controls (906 alleles of each) is sufficient to detect association with 80% power (5% level) if allele frequencies are .15 and .20.

A final point is that all trios, singleton cases, and singleton controls can be analyzed simultaneously, thereby increasing power and providing independent data sets for confirmation.

B. Eligibility Criteria, Definitions and Exclusion Criteria

Eligible for the study are men and women with type 1 diabetes, aged 18-54 at the time of enrollment, living in the U.S.A. or Canada. The JDRF collection will include all volunteers, regardless of race or ethnic origin. However, the size of the proposed data collection for this study will not be large enough to support meaningful subgroup analyses based on ethnicity. (Sample size requirements range from ~166 ethnically-matched trios for the most optimistic cases to several hundred trios for more typical scenarios. We estimate having a heterogeneous sample of minority families comprising, in total, only about 60 trios. The prognosis for controls trios and singleton cases/controls is even worse since the former typically convey less power than case families and the latter are highly susceptible to the type of admixture likely to be found in a heterogeneous group.) Data on subgroups will be collected and stored, and the Coordinating Center will monitor and develop opportunities to utilize this information. As many total examinations as necessary to collect the 4300 samples for comparison with the BDA data collection will be made.

B.1 Definitions for inclusion into the study

Probands for this data collection must have type 1 diabetes and either presence of absence of diabetic nephropathy according to the following definitions.

Type 1 diabetes is diagnosed if:

- Subject had diabetes diagnosed before age 31
- Treatment with insulin was instituted within one year of diagnosis, and
- Treatment with insulin has been uninterrupted since diagnosis

Presence of diabetic nephropathy is diagnosed if:

Subject with diabetes for at least 10 years has persistent proteinuria or ESRD (not due to condition other than diabetes)

Persistent proteinuria is defined as at least 2 out of 3 tests positive for albuminuria (at least 1 month apart), i.e., dipstick (Albustix or Multistix) at least 1+ or ACR value exceeding 300 μg albumin/mg of urine creatinine.

Absence of diabetic nephropathy is diagnosed if:

Subject has persistent normoalbuminuria despite duration of type 1 diabetes for at least 15 years and has never been treated with ACE inhibitors.

Persistent normoalbuminuria is defined as at least 2 out of 3 ACR measurements (at least 1 month apart) in random urine specimens being less than 20 μg of albumin/mg of creatinine. If 3 ACR measurements are needed, the highest must also be less than 40 μg of albumin/mg of creatinine.

(See Appendix 3 for justification).

B.2 Definitions for exclusion from the study

Individuals will be excluded from the study if they do not meet the inclusion criteria just described or if any of the following exclusion criteria are met:

- Unable or unwilling to give informed consent
- Unable to communicate with staff
- Major psychiatric disorder such as schizophrenia
- Exclusion in relation to medication
  - Any antihypertensive medication for controls
- Infectious disease
  - Self-reported HIV positivity
  - Active tuberculosis
- Other kidney disease in cases
  - Alport syndrome
  - Analgesic nephropathy
  - Atheroembolic renal disease
  - Congenital nephrotic syndrome
  - Focal segmental glomerulosclerosis (FSGS)
  - Glomerulonephritis
  - Goodpasture's syndrome
  - HIV nephropathy
  - IgM mesangial proliferative nephritis
  - Lupus nephritis
  - Kidney cancer
  - IgA nephropathy
  - Polycystic kidney disease
  - Urinary tract infection
    (Note: infectious processes such as cystitis and urinary tract infections do not represent a permanent exclusion. Patients may be recontacted or asked to send a supplemental urine sample at a later date.)
- Pregnant women (although they may be reconsidered 3 months after delivery)

C. Patient Acquisition

Half of the index patients and half of the parents will be recruited and examined at Joslin Diabetes Center in Boston and half will be recruited by Matthews Media Group in conjunction with The George Washington University Biostatistics Center and EDIC centers (excluding centers in New England and Eastern Canada) and renal centers nationwide. The following is a description of the identification, recruitment, and examination of index subjects (and parents) for the study. Since these activities are significantly different for the Joslin Diabetes Center and for The George Washington University Biostatistics Center, they will be described in detail separately.

C.1 Patient acquisition at Joslin

C.1.1 Identification of patients

The patients who will be contributed by the Joslin Diabetes Center to this project will be recruited from among those already participating in (or being recruited for) our protocol (Joslin CHS # 87-10) "Genetic Factors in the Late Complications of Diabetes: Family Studies". The patients will be invited to participate in both protocols and will be asked to sign both consent forms if they agree to both. Patients are recruited for that study through the Joslin Clinic Internal Medicine Units and the Renal Unit. We also send invitation letters to former campers and advertise on the Internet and on Joslin bulletin boards. An additional source for nephropathy cases will be dialysis centers in New England and Eastern Canada.

C.1.2 Recruitment of patients

The study coordinator will call potential candidates meeting the study criteria. During the conversation, the coordinator will explain the study, its goals, the protocol, potential uses of results, and participant compensation. The coordinator will also answer any questions the candidate may have. If patient agrees to participate, Coordinators schedule an exam with the participant in one of three ways (depending on what the participant prefers):

- At the Joslin Clinic. This can be done either in conjunction with another appointment or for the sole purpose of participating in the study.
- At the participant's home. This is the usual option for participants who are not current Joslin Clinic patients, but who live in New England.
- Via mailers that the participant completes at his/her local lab or doctor's office. This is the option for participants who are not current Joslin Clinic patients and live outside of New England, or for those who prefer the convenience and comfort of participating at their own doctor's office.

C.1.3 Examination of patients

C.1.3.1 Examination of patients at Joslin Clinic:

A recruiter will review the consent form with the patient. If the patient agrees to participate and informed consent is obtained, the recruiter will administer a questionnaire to assess medical history, medication usage and family history of diabetic nephropathy. The recruiter will also take two seated blood pressure measurements from the same arm using a standard protocol. The recruiter will also record the patients pulse, draw blood, and instruct the patient to leave a urine sample. Because appointments are scheduled throughout the day, this will be considered as a random urine sample. The blood and urine samples will be used for determining HbA1c, lipids, serum creatinine, and serum cystatin, and for DNA extraction and lymphocyte cryopreservation. Urine and serum will also be stored for further investigation.

C.1.3.2 Home visits

Other than the fact that recruiters meet patients at their home at a day and time most convenient for the patient, home visits are virtually identical to Joslin Clinic examinations. One slight difference is that aneroid as opposed to mercury sphygmomanometers will be used for home visits. To ensure compatibility, aneroid sphygmomanometers will be calibrated before each visit.

C.1.3.3 Mailers

For patients participating by mail, the first step is to review the consent form and questionnaires via telephone. Then, a recruiter will send mailers to participants containing all questionnaires, blood and urine sample collection tubes, instructions for participant and lab, doctor's order, billing and shipping info, and 2 consent forms (one to return, one for participant to keep). The recruiter will then call the lab to explain procedures for performing the blood draw, and then make appointments for study subjects (if applicable). The participant will complete both the consent form and questionnaire, and return them to the Joslin Clinic in included self-addressed, stamped envelope. Blood/urine collection and pulse/blood pressure measurements are done at lab of participant's choice, adhering to our standard protocol. Follow-up calls to the subject are made if completed kits are not received in a reasonable amount of time. Once the samples are collected, the lab repacks the kit, and either drops the completed kit (with FedEx form already filled out by the study coordinator) in a FedEx drop box, or calls coordinator to arrange for courier retrieval. If additional information is needed to complete the exam, medical records of Joslin Clinic patients can be abstracted. Participants who are not Joslin Clinic patients may complete Medical Record Release forms to grant access to medical records from other sources. Urine and serum will not be stored for those participating by mail.

C.1.3.4 Results and follow-up

Recruiters are responsible for submitting vouchers for monetary compensation in a timely fashion. Results (urine albumin/creatinine ratio, A1c, and lipids) are sent to subjects and their doctors (if requested) within 2 weeks of participation. A "thank you letter" is sent with the results.

C.1.4 Data forms

A set of standard data forms will be developed and included as part of a manual of operations.

C.2 Patient acquisition at The George Washington University Biostatistics Center

C.2.1 Identification and recruitment of cases and controls

Matthews Media Group (MMG) will provide recruitment support for this project and will work closely with The George Washington University Biostatistics Center to identify the potential cases and controls. MMG will recruit outside of New England among the JDRF community, Participating EDIC sites, the diabetes camping community, and other diabetes communities as appropriate. The camps are members of the Diabetes Camping Association and are operated independently or sponsored by hospitals or non-profit institutions. MMG will send promotional materials to the diabetes camp staffs in the U.S. and Canada, requesting that the GoKind Study materials be made available to camp counselors over age 18, and also to parents on drop-off or pick-up day. Those camps which maintain current addresses of former campers will be approached and asked to send a camp mailing with GoKind Study brochures to previous campers who may be eligible to participate in the study. Recruitment also will focus on renal care centers and intermediary groups concerned with kidney disease. And, MMG will publicize the study through general and scientific media.

MMG's call center will employ a toll-free number, respond to inquiries and contact family members, once permission to contact all family members has been obtained from the individual family members. Prospective participants and other callers will speak with a trained information specialist if they call during regular business hours. Calls at other times will be returned the next business day.

When the initial family member arrives at the screening site, the recruiter will provide that family member information about the study for the remaining two family members. MMG and GoKinD sites will only contact family members once these family members have indicated an interest in the study.

C.2.2 Examination of patients at Participating sites

Cases and controls will be screened at Participating EDIC sites for persistent proteinuria by repeated random urine collections. The cases and controls will be seen by the staff of the EDIC site to give informed consent, complete a medical history, have physical examinations performed, provide specimens for HbA1c, lipids, serum creatinine, serum cystatin, and DNA extraction and lymphocyte cryopreservation. (When possible, urine and serum will also be stored for further investigation). All cases who are qualifying for the study on the basis of persistent proteinuria will be labeled temporarily eligible until at least 2 out of 3 (a month apart) urine ACR measures are > 300 μg albumin / mg creatinine. These cases will be supplied with urine collection kits and mailers. They will be instructed to mail to the Central Biochemistry Laboratory another urine collection a month from the initial collection.

C.2.3 Mailers

For patients participating by mail, the first step is to review the consent form and questionnaires via telephone. Then, staff will send mailers to participants containing all questionnaires, blood and urine sample collection tubes, instructions for participant and lab, doctor's order, billing and shipping info, and 2 consent forms (one to return, one for participant to keep). Staff will then call the lab to explain procedures for performing the blood draw, and then make appointments for study subjects (if applicable). The participant will complete both the consent form and questionnaire, and return them to The George Washington University Biostatistics Center in the included self-addressed, stamped envelope. Blood/urine collection and pulse/blood pressure measurements are done at lab of participant's choice, adhering to the standard protocol. Follow-up calls to the subject are made if completed kits are not received in a reasonable amount of time. Once the samples are collected, the lab re-packs the kit, and either drops the completed kit (with FedEx form already filled out by the study coordinator) in a FedEx drop box, or calls coordinator to arrange for courier retrieval. If additional information is needed to complete the exam, follow-up calls will be made, as needed can be abstracted. Participants may complete Medical Record Release forms to grant access to medical records from other sources.

C.2.4 Data forms

A set of standard data forms will be developed and included as part of a manual of operations.

D. Specimen Handling and Inventory

The University of Minnesota's Department of Laboratory Medicine and Pathology will serve as the Central Biochemistry Laboratory (CBL) for this project. The Biostatistics Center and the CBL have collaborated on several major studies since 1982, and this long-term collaboration guarantees the documentation of receipt, accession, processing and analysis of constituents in the specimens. The CBL will receive all specimens, perform all biochemical measurements, cryopreserve and transform lymphocytes, and prepare a stable cell lysate from whole blood for DNA isolation using the Gentra Purgene protocol. The CBL will ship transformed lymphocytes and stable cell lysates to CDC. CDC will isolate DNA from the cell lysates and prepare it for storage. CDC will track and maintain the inventory of the DNA and transformed lymphocytes. Procedures for specimen handling are included in Appendix 1. The

Specimen Handling Committee will oversee issues related to specimen handling, inventory, and distribution.

E. Data Management

Data management will encompass a variety of activities taking place at each of the institutions comprising the Coordinating Center. Ultimately, The Biostatistics Center at George Washington University will be responsible for managing the data resource for this project. However, significant data management will also occur at Joslin, CDC, and the CBL. Once participating scientists begin to use DNA from this resource, another aspect of data management will also involve cataloging and storing genotypic data returned to the Coordinating Center. This introduces a number of special challenges, which are briefly outlined below.

E.1 The George Washington University Biostatistics Center

The collaborators will be responsible for weekly mailing to the Coordinating Center and timely shipments to the CBL. They will be responsible for responding to the data queries.

Weekly data are mailed from the collaborators and transmitted from the CBL to the Coordinating Center. Data management is conducted using Visual, FoxPro Version 5 software and SAS. Incoming data forms are keyed into a separate FoxPro data file for each form. Preliminary edit checks are conducted during data entry and extensive edit programs are run after data entry is completed, generating an edit flag file as well as an edit report to be sent to the collaborator. Newly entered forms are merged into the master. These are converted to SAS data sets for analysis and reports.

Data that are transmitted from the CBL and CDC will be uploaded to the Coordinating Center's computer system. These data will be examined for missing, out-of-range, and inconsistent values. If problems are detected, the source of the data will be contacted for resolution.

George Washington's Coordinating Center's computer facility is a multi-platform consisting of UNIX RISC workstations, personal computers and an IBM 4381 midrange system. The 4381 serves as a central data management system, with Ethernet links to the other platforms and to the Internet global network.

All data forms collected during the term of GoKinD will be microfiched. Those forms that are microfiched will be destroyed eventually.

E.2 Joslin Diabetes Center

The Joslin Diabetes Center will collect data on over 2,000 individuals. Data will be entered into Microsoft Access by patient recruiters. Joslin's data manager will be responsible for designing this data entry system, which will include a relevant set of data checks to help recruiters detect problematic data immediately. Each week, this data will be exported from this PC program to a Unix-based SAS data set and sent, via ftp, to The George Washington University Biostatistics Center. Joslin's data manager will oversee the data conversion and also test the data for quality and reasonability before sending it. Once The George Washington University Biostatistics Center merges this information with data from other collaborative sites and performs final validation and error checking, a complete data set will be sent back to Joslin for storage and various analyses described elsewhere. To guarantee reliability, expandability, and security, Joslin will store data on a Sun Ultra 10 workstation. Access to this workstation will be restricted by password and data backups will occur at regular intervals.

E.3 Centers for Disease Controls and Prevention (CDC)

CDC will serve as the permanent storage facility for the DNA and the transformed cells. CDC will also store a copy of the entire database coded without personal identifiers. CDC will access and utilize the database under the same guidelines as the Coordinating Center.

E.4 Central Biochemistry Laboratory (CBL)

Research GoKinD laboratory data will be stored on the Oracle database dedicated to research data storage at the CBL. Data will be extracted from the CBL research location and transmitted via FTP weekly to the Coordinating Center.

E.5 Database of genotypic results

A condition for access to the GoKinD DNA resource is that all genotype data produced must be returned to the Coordinating Center. Development of a useful system to store such data is clearly important. A consistent, standardized nomenclature for the genotype data is needed and this nomenclature will need to include as much available information as possible. Every effort will be made to adopt standards compatible with those promulgated by the National Center for Biotechnology Information (NCBI), to facilitate any further analyses of data in the JDRF resource. It is anticipated that genotype information will include, but may not be limited to, SNPs, microsatellites, RFLPs, gene mutations, polymorphisms, and chromosomal location. The Executive Committee will continue to address this area in years 1 and 2 of the initial funding period.

E.6 Data security and backups

Rigorous procedures for data security and data backups will be instituted for the GoKinD study.
Electronic data will be password protected and hard copies will be stored in locked file cabinets. As an additional precaution, neither the CDC nor the CBL will have access to personal information such as name, address or social security number. Likewise, DNA samples sent to participating scientists will be referenced only by a coding scheme, since personal information will not be made available.

The Biostatistics Center will perform data backups using a standard protocol successfully used in other similar projects. An offsite copy of the database will also be stored at CDC and at Joslin.

Detail procedures for data security and data backup will be documented in a manual of operations.

F. Web Usage

CDC will prepare a catalog of available DNA and the Coordinating Center will participate in publicizing the availability of the genetic specimens and clinical data. The Coordinating Center will also create an Internet-based system that will serve as the central avenue for communication with the participating scientists. This Internet site will reside on a secure Sun workstation to be located at Joslin and will use the full functionality of hypertext machine language (HTML) to provide up-to-date information regarding the families that are available and the markers that have already been genotyped by other researchers. The web site will also include information about how to obtain phenotypic and genotypic information and will provide a convenient link for users to provide feedback. An important feature will be a comprehensive list of changes made to any data that have already been released. In this way, users can simply update their own data while avoiding the confusion of having multiple

versions of the data sets. Once investigators have completed their genetic analyses, they will be expected to return the results to the Coordinating Center (George Washington University), where a database of results will be maintained. This database will be advertised on the web site. If feasible, the web site will also contain abstracts of work performed using the resource.

The specific content for the project's web site will be developed during years 1 and 2. Joslin's genetic epidemiologist will be responsible for developing various standards for the web site (e.g., nomenclature for genetic polymorphisms). Depending on the scope of the web site, help from outside the Coordinating Center may be required.

G. Genetic Epidemiologic Analyses

The Coordinating Center will also conduct baseline studies to aid investigators in assessing the usefulness of the collection for studying various aspects of diabetes and its late complications. Preprints of manuscripts reporting these results will be made available to interested participating scientists, and general information about the resource will be accessible on the Internet.

G.1 Epidemiologic analysis

To assist investigators in assessing the usefulness of this resource for specific studies of late diabetic complications, a variety of epidemiologic analyses will be carried out by the Coordinating Center. Emphasis will be placed on the following four complications, which will be studied using phenotypic data collected on cases, controls, and parents:

Diabetic nephropathy
Discrete: absence or presence of advanced nephropathy, either proteinuria or ESRD
Continuous: serum creatinine at the time of examination, serum cystatin at the time of examination, age at onset of proteinuria or ESRD, rate of declining renal function

History of proliferative retinopathy
Discrete: present or absent ophthalmologist diagnosis

History of coronary artery disease
Discrete: composite index including angina, MI, angioplasty, or bypass

Hypertension
Discrete: composite index including doctor's diagnosis, history of antihypertensive treatment, and blood pressure measurements at the time of examination

In the analyses, the following covariables will also be considered:

Recruitment-related
- Mechanism of recruitment
- Center of recruitment
- Socio-economic status
- Education

Diabetes-related
- Age-at-onset of diabetes
- Duration of diabetes
- History of health care utilization

Family-history

- Self-reported race
- Date of birth of parents, and date of death if applicable
- Place of birth for all four grandparents and their ethnicity

These analyses will provide investigators with information necessary to avoid performing inappropriate studies and to minimize sampling bias. It will also serve as a resource for determining optimal study designs and for identifying particular groups whose characteristics might expedite the discovery of genes involved in diabetes and its complications.

The scope of the epidemiologic analysis and the detailed plans for implementation will be developed at the Joslin Diabetes Center. Then, under the supervision of Dr. Rogus, the work will be carried out by Joslin's genetic epidemiologist and, where appropriate, by George Washington's statistician.

CDC, the Coordinating Center, and collaborators will publish analyses of the HLA and other genotype data that it provides as it relates to the phenotypic data in the database.

G.2 Basic statistical genetics

The proposed data collection will contain a rich set of phenotypic endpoints collected for both population-based and family-based sampling units. This variety will offer limitless opportunities for the study of diabetes and its complications. However, not all study designs will take full advantage of the available data and, more importantly, some designs can produce results that are severely biased. Although **it is not the intention of the Coordinating Center to regulate the designs used by participating scientists**, we will undertake certain foundational work for the benefit of those researchers who would like some basic guidance. Results of this work will be submitted for publication for widespread distribution and also made available in advance of publication through preprints. Information will also be made available to researchers on the project's web site.

One area that should be explored is the impact of incomplete nephropathy families. As mentioned elsewhere, although the Coordinating Center will make every effort to collect bona fide trios, families with missing parents may need to be substituted in some instances. If this concession is made, the initial work must focus on the best types of families to collect, given the characteristics (e.g., prevalence) of diabetic nephropathy. Prioritization of family types without sufficient investigation into the relative loss of power would be irresponsible. Once the prioritization is finalized, additional work will be done to suggest efficient ways in which investigators may wish to use these incomplete families in genetic studies. This work, which will be done primarily by Joslin's genetic epidemiologist, will benefit from Dr. Rogus's role as co-investigator on an NIH-funded project to develop statistical methods suitable for this type of family collection.

In addition to these design issues, we will assemble an informational resource relating to a number of other pertinent issues. These will include methods for dealing with segregation distortion and strategies to avoid misinterpreting the effects of a type 1 diabetes genes. Other issues such as survival bias, data censoring, and qualitative vs. quantitative analysis may also be addressed.

The statistical genetics work proposed in this section represents only a small fraction of what needs to be done to mine this resource most efficiently. Participating scientists will, therefore, also be encouraged to develop appropriate methodology. Moreover, researchers at Coordinating Center institutions, in particular the Joslin Diabetes Center and George Washington University, will look for additional opportunities to submit proposals to the JDRF.

H. Follow-Up

We plan to follow patients every three years to evaluate 1) who is still alive, 2) which patients with normoalbuminuria progress to microalbuminuria or more advanced kidney disease, 3) which patients with proteinuria progress to ESRD, and 4) rate of renal function decline based on serum creatinine and serum cystatin. This activity will be contingent on additional funding from the JDRF. Patients will be identified through record locators stored at the Biostatistics Center in a file apart from study data. It is expected that if this plan is adopted that the local clinics will implement it. Furthermore, patients will be contacted once per year by postcard for the purpose of obtaining current address information. Matthews Media Group will coordinate the annual contact.

Planning for the first round of follow-up will occur in the fourth year of the initial funding period. At this time, logistics will be finalized, and other relevant endpoints and ancillary studies (which may require additional consent) may be considered.

I. Policy and Organization

The JDRF Coordinating Center for Studies of the Genetics of Type 1 Diabetes and Its Complications (funded by the JDRF) is responsible for recruiting participants and collecting samples. This Coordinating Center consists jointly of the Joslin Diabetes Center investigators (John Rogus, local PI, Andrzej Krolewski and James Warram, coinvestigators, 9 full time, 3 partial time) and George Washington University Biostatistics Center (GWUBC, Patricia Cleary, local PI, 2 full time, 6 partial time) with the University of Minnesota serving as the Central Biochemistry Laboratory (Michael Steffes, local PI, 10 partial time, depending upon rotation). Joslin Diabetes Center and GWUBC each are responsible for recruiting one half of each category of the participants (e.g., 300 case trios, 250 control trios, 250 cases, and 250 controls). The Joslin Diabetes Center recruits participants directly. GWUBC 1) recruits participants through the EDIC associated centers (approximately 27 Centers with appropriate manpower at each) and renal care centers (to be determined), and 2) is responsible for the contract with Matthew Media Group (Therese Gibson, local contact, 2 full time, 6 partial time) which provides recruitment support. The University of Minnesota as the Central Biochemistry Laboratory is responsible for receiving and processing samples from all centers, providing biochemical tests, transforming lymphocytes, and preparing stabilized cell lysates for DNA extraction. The National Diabetes Laboratory of CDC (Patricia W. Mueller, local PI, Suzanne Cordovado, coinvestigator, 6 full time, 2 partial time 1) will isolate the DNA and perform the initial genetic analyses as specified in the protocol, 2) will store the transformed cell lines and DNA aliquots at CASPIR through the contract with ATCC, 4 partial time employees as needed, using 12 of the total 153 liquid nitrogen freezers for the approximately 320,000 aliquots of cell lines and DNA. The JDRF oversees the project with direct staff involvement, a Steering Committee, and the JDRF Research Advisory Board, consisting of scientific and lay members.

This section describes funding mechanisms, organizational units, committees, and policies. In addition, an organizational chart for the GoKinD study can be found in Figure 1.

I.1 Funding Mechanism/Study Resources

Funding for the coordinating center and the CBL is provided by the JDRF. All supports for the Collaborations will be provided through the Coordinating center. CDC is funded by congressional appropriations.

I.2 Policies

I.2.1 Institutional Review Board (IRB)

Ethical approval for the collection, storage and analyses of DNA must be obtained from each of the organizational units prior to the first participant visit. Each organizational unit except JDRF must have either a Multiple Project Assurance (MPA) or a Single Project Assurance (SPA) issued by the National Institutes of Health (NIH) Office for Protection of Research Risks (OPRR).

I.2.2 Publication Policy

In order to realize maximum value from the JDRF GoKinD collection, peer-reviewed publications of analyses of data and findings will be strongly encouraged. A primary avenue for dissemination of information will be from participating scientists using the resource. Those scientists who receive DNA and generate genetic data will be encouraged to publish the results of their analyses in a timely manner, and their right to priority in publication of the data they contribute is recognized.

The Coordinating Center will publish on the characteristics of the study population, including a baseline publication describing the resource. The Executive Committee will organize a writing group for the baseline publication. This group will consist of the Principal Investigators of the Coordinating Center, who will serve as the first two authors, and others who may or may not be part of the Executive Committee. The Executive Committee will coordinate the efforts of the writing group, establish priorities for data analysis by the Coordinating Center, handle authorship issues, and help edit the manuscript. Both the Executive and Steering Committees will review the baseline publication to ensure proper representation of the resource.

All publications containing data from the JDRF resource will be required to cite the baseline publication and acknowledge the Coordinating Center. The Coordinating Center investigators expect authorship only when they have made a substantial contribution to the research. Likewise, when contributed genetic data is used in analyses by others, the contributors should receive authorship only if they contribute substantially to results.

A Publications Oversight Committee will be established with members appointed by the JDRF to adjudicate publications disputes, establish such additional publications guidelines as may be needed, and to ensure the timely publication of results.

I.2.3 Protocol Changes

The objectives of the GoKinD study are most likely to be achieved if the Protocol is not altered. Any changes in the Protocol may complicate the collection of specimens and data and will cause communication difficulties among the collection sites. If, however, the Executive Committee considers a protocol change necessary, the change must be approved by all members of the Committee before it is forwarded to the Steering Committee.

I.2.4 Internal Monitoring

Mechanism will be instituted for continuous performance monitoring of all GoKinD study units. Recruitment goals will be established by the Executive Committee for the Coordinating Center and the Executive Committee will review performance on a monthly basis. External quality control surveillance will be instituted to assess the precision of all measurements and procedures performed by the Central Biochemistry Laboratory (CBL). The quantity and quality of the DNA and the storage location and the performance of the CBL with respect to freezers malfunction will be reviewed by the Specimens Handling Committee (SHC)

regularly. The Center for Disease Control will also report to the SHC on the status of specimen stored in that facility.

The Executive Committee will site visit the Coordinating Center (Joslin & GWU), the CBL and the CDC during the first year of the study and may revisit again if there is the need to do so.

I.2.5 Sample Availability

Once 40% of the case trios have been collected, the Executive Committee will convene a meeting to discuss making samples available. Based on preliminary discussions, it is anticipated that the first half of the case trio collection will be prepared for distribution as soon as all 300 trios are available. A similar scheme is expected for control trios and for cases and controls.

I.3 Organizational Units

The GoKinD study is the first project carried out by the JDRF Coordinating Center for Studies of the Genetics of Type 1 Diabetes and its Complications. Below is a description of each organization unit of the Coordinating Center and its related oversight committees. An organizational chart for the GoKinD study can be found in Figure 1.

I.3.1 Juvenile Diabetes Research Foundation (JDRF)

The JDRF Coordinating Center and the GoKinD study are underwritten by funds from the JDRF. A member of the JDRF staff serves as a liaison to the Coordinating Center.

I.3.2 Coordinating Center

The JDRF Coordinating Center for Studies of the Genetics of Type 1 Diabetes and its Complications ("Coordinating Center") is a joint collaboration between the Joslin Diabetes Center and The George Washington University Biostatistics Center. The Coordinating Center is responsible for 1) logistics, including but not limited to identifying and recruiting affected family trios, control family trios, and individual cases and controls; 2) specimen and record labeling, handling, and shipping to CDC; 3) database management of the phenotypic and genotypic data; 4) maintenance of inventories of all genetic specimens; 5) updating the database with the results of genetic analyses; 6) analysis of the epidemiologic characteristics of the study population; and 6) development and implementation of strategies to maintain contact with the cohorts in order to secure more than 80% follow-up. The Coordinating Center is responsible for the protocol, manual of operations, and data collection forms development. The Coordinating Center will monitor protocol performance and provide the Steering Committee with regular updates on the status of the study.

I.3.3 University of Minnesota (CBL)

The laboratory at the University of Minnesota will provide eligibility, baseline and repeated measures of HbA1c and risk factors for nephropathy and cardiovascular disease. The CBL will receive serum, whole blood and urine for the measurement of lipid profile, creatinine and cystatin in serum, HbA1c in whole blood and albumin and creatinine in urine. The one 10-mL tube of EDTA whole blood will be used to prepare stable cell lysates for DNA extraction at the CDC. The CBL will also prepare two whole blood CPT samples for the cryopreservation and transformation of peripheral blood lymphocytes into lymphocyte cell lines. These will be shipped to the CDC for storage.

All results will be transmitted to the Coordinating Center weekly.

I.3.4 United States Centers for Disease Control and Prevention (CDC)

The CDC will be the principal storage facility for the DNA samples and cell lines. CDC will track and maintain the inventory of the DNA and cell lines and will be responsible for disbursement of samples to approved investigators.

The CDC will perform HLA genotyping for DR-B1 (including DR-4 subtypes), DQ-B1, and DQ-A1 alleles, in addition to *INS* SNP characterization. They will type 4 microsatellite markers to test for sample mix-ups and non-paternity. CDC will also consider genotyping the collection for additional IDDM loci when good candidate genes for genetic risk at the individual loci are identified with approval of JDRF. CDC may submit additional genotyping proposals through the regular process that will be established by JDRF. CDC will report all genotyping results to the Biostatistics Center for incorporation into the database.

A copy of the study's database (without information such as name, address, and social security number) will also be sent to the CDC for backup and updated periodically. CDC will access and utilize the database under the same guidelines as the Coordinating Center.

I.3.5 Clinical Centers

EDIC clinical centers, Joslin Diabetes Center, and additional renal care sites will implement the GoKinD proposal.

I.3.6 Matthews Media Group

Matthews Media Group will help recruit participants for GoKinD sites overseen by The George Washington University Biostatistics Center. MMG will coordinate these activities through JDRF and with the Joslin Diabetes Center, which will recruit in New England and Eastern Canada.

I.4 Committees

I.4.1 Executive and Steering Committees

The day-to-day operations of the Coordinating Center will be the responsibility of the Coordinating Center Executive Committee. Specific areas of responsibility include recruitment and retention, data management and transfer, and communications. This committee will consist of the Principal Investigators of the Coordinating Center, a representative of the United States Centers for Disease Control and Prevention (CDC), the Director of the Central Biochemistry Laboratory (CBL), a representative from Matthews Media Group (MMG) and a representative from JDRF.

The policies, procedures and projects of the JDRF Coordinating Center for the Study of the Genetics of Type 1 Diabetes will be directed by a Steering Committee composed of members appointed by the JDRF, and consisting of scientific and lay reviewers. Steering Committee members serve four-year terms.

The Principal Investigators of the Coordinating Center will be invited as observers to meetings of the Steering Committee.

I.4.2 JDRF Research Advisory Board (RAB)

The Steering Committee serves as an advisory committee to the JDRF Research Advisory Board (RAB), which will have final overall responsibility for the Center.

The RAB is a committee comprised of scientific and lay members who advise the JDRF Board of Directors regarding all research matters of the Foundation. This includes all

policies and procedures related to the research review process, overseeing the activities of its review subcommittees (Medical Science Review Committee and Lay Review Committee), evaluating research progress, and examining research opportunities that are commensurate with the JDRF mission.

I.4.3 Specimen Handling Committee (SHC)

The SHC, including a CBL representative, a CDC representative, a JDRF representative, two members of the Steering Committee, and the Principal Investigators of the Coordinating Center, will oversee issues related to specimen handling, inventory, and distribution. Currently, this committee includes Drs. Steffes, Mueller, Nierras, Bain, Rich, Cleary, and Rogus. Dr. Mueller is currently serving as the committee chair.

I.4.4 Application Review Committee (ARC)

JDRF reserves the right to review all proposals for use of the data and/or samples in all JDRF resource collections. Successful applicants:

- must provide information derived from the samples and the data to the Coordinating Center;
- must notify JDRF of papers accepted for publication; and
- must acknowledge JDRF and Coordinating Center support in such papers.

JDRF will appoint an Application Review Committee (ARC) to expedite review of proposals for use of the GoKinD DNA resource, according to JDRF's established review procedures.

I.4.5 Publications Oversight Committee (POC)

A Publications Oversight Committee will be established with members appointed by the JDRF. Its duties will be to encourage publication of analyses of data in keeping with the Publications Policy. These duties will include the adjudication of publications disputes, establishment of such additional publications guidelines as may be needed, and the assurance of the timely publication of results.

J. Timeline for the collection of families and other project activities

As the collection of 600 nephropathy families is likely to represent the primary bottleneck in assembling this resource, nephropathy families will be aggressively pursued in the first two years of the initial funding period. Although every effort will be made to adhere to the collection of bona fide nephropathy trios, an alternative scheme allowing for the collection of some families with missing parents will be available. If this "plan B" is activated, the Coordinating Center will take great care to preserve statistical power and to alert users about the potential limitations of the incomplete families. Collection of control trios will begin near the end of Year 2 and will continue into Year 4. The majority of singleton cases and controls will be collected in Year 4.

Activities also occurring in Year 1 will include creation of data entry systems, phenotypic databases, and systems for (with quality control testing) for data transfer. Maintenance of these systems will be required throughout the entire funding period. Epidemiologic and foundational statistical genetics work will also be conducted throughout all four years.

The system to manage genotypic data will be developed over the first three years and will be tested in Year 4. Early design issues will include adopting a standard nomenclature and devising a system to deal with missing/duplicated data.

K. Aspects related to human subjects and the consent process

There are three main issues related to human subjects and the consent process. Each one of these will now be described.

K.1 Information given to potential subjects regarding the consequences of their participation

Information on the consent form is the usual instrument for informing subjects about the procedures they will be exposed to as a participant, the risks associated with these procedures, and benefits that subjects can expect to realize from participation. In order to keep the format and content of the consent form simple and direct, an orientation brochure can be designed to supplement the consent form and elaborate on some of the points to improve their understanding. Such material and any revisions are subject to IRB review. A draft consent form is included as Appendix 2.

K.2 Measures that will be undertaken to protect the confidentiality of individual information

Confidentiality of subject information is a major concern of the participating individuals and the organizations that oversee the use of human subjects in research and seek to set standards for that research. The concern is not only that personal information will get into the hands of third parties, such as employers and insurance companies, but also that genetic information may get into the hands of individuals who cannot benefit from the knowledge but can be harmed by it. Three types of data can be distinguished by their sources: data identifying individuals and characterizing their phenotypes that are collected by centers recruiting subjects; phenotypic data generated by the central laboratory; and genotype data generated by investigators using the DNA. The following policies will be implemented to protect confidentiality of this data. 1) Samples sent to CDC and the CBL will be coded to track samples but will not contain identifiers such as name, address, or social security number. 2) Databases compiled during the recruitment process will be password protected. 3) Recruitment forms will be kept in locked file cabinets. 4) Identifying information maintained by the Biostatistics Center will not be stored in the same place as other study data.

Special attention must be given if participants are contacted while at work. Should a participant wish to be contacted at the workplace, several precautions must be taken. First, it is important to maintain confidentiality with respect to any information concerning the study (including the fact that a person is being asked to participate in the first place). To do so, the phone conversation will begin by the recruiter simply asking to speak with the potential participant. If he/she is not available, the recruiter will provide no additional information and will simply say that he/she will call back later. If the recruiter is successful in reaching the participant, he/she will ask the participant if it is a good time to speak. Finally, if the participant becomes uncomfortable at any time during the call, alternative arrangements will be made.

K.3 Information given to participants regarding their genotype and overall project outcomes

Study participants will receive the results of the standard tests on their blood and urine. They will <u>not</u> receive information about the results of studies of their DNA. All patients with diabetes may benefit if this project is successful in discovering the genes responsible for susceptibility to diabetic kidney disease. At some time in the future, there may be justification for informing the patients of the results of the tests of their DNA. The JDRF and the Steering

Committee will have an ongoing responsibility for evaluating whether the patients would benefit from that information and, if they decide there is sufficient justification, they will forward a specific proposal to the IRBs at institutions that provided patients.

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The Diabetes Control and Complications Trial Research Group. 1997. Clustering of long-term complications in families with diabetes in the Diabetes Control and Complications Trial. Diabetes 46:1829-1839.

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Lunetta KL, Faraone SV, Biederman J, Laird NM. Family-based tests of association and linkage using unaffected sibs, covariates, and interactions. American Journal of Human Genetics 66:605-614.

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Spielman RS, McGinnis RE, Ewens WJ. 1993. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). American Journal of Human Genetics 52:506-516.

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Allison DB. 1997. Transmission-disequilibrium tests for quantitative traits. American Journal of Human Genetics 60:676-690.

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Morton NE, Collins A. 1998. Tests and estimates of allelic association in complex inheritance. Proceedings of the National Academy of Sciences of the United States of America 95:11389-93.

APPENDIX 1: PROCEDURES FOR SPECIMEN HANDLING

A1.1 Blood Collection Tubes

About 35 mL of blood is drawn from each participant using four blood collection tubes. Samples from these four tubes will be used to prepare lymphocyte cell lines, isolate DNA, estimate biochemical measurements and be maintained in long term storage. The collection tubes are provided to the clinical centers by the CBL.

Tubes #1 and #2 are 8-mL black and blue-topped tubes (CPT) containing an anticoagulant and a cell-separating reagent. After each tube is filled with blood, they are inverted eight times then placed into a room temperature rack. These tubes are not centrifuged or refrigerated.

Tubes #3 is a lavender-topped 10-mL tube containing the liquid anticoagulant EDTA. After the tube is filled with blood, it is inverted eight times then placed into a room temperature rack.

Tube #4 is a 10-mL red and gray-topped tube. This tube does not contain anticoagulant, so it does not need to be mixed following collection. After drawing, the blood is allowed to clot at room temperature for 30 minutes.

Blood Collection Tube Processing

The collection tubes and the plastic storage microvials are labeled prior to the blood collection by the clinical center with pre-numbered adhesive labels provided by the CBL.

At the conclusion of venipuncture, all the tubes remain at room temperature.

Tubes #1 and #2 remain in a rack at room temperature. The tubes remain sealed to preserve sterility. These tubes are not centrifuged or refrigerated. They are to remain at room temperature until shipment to the CBL for lymphocyte cryopreservation and transformation. Shipment of these tubes must occur on the day of specimen collection.

Tube #3 is inverted four times before removing the lavender stopper. Using a graduated plastic transfer pipette, 0.5 mL of whole blood is aliquotted into a 2 mL microvial that is then capped and stored in the refrigerator until shipment to the CBL for hemoglobin A1c assay. The lavender top is replaced on tube #3. The remaining blood in tube #3 is centrifuged at 3000 rpm for ten minutes. After centrifugation, using a plastic transfer pipette and being careful not to disturb the cell layer, the three equal aliquots of plasma are carefully transferred into three microvials. These vials are capped and stored in the freezer until shipment to the CBL for temporary storage at -70°C. The original tube #3 with the remaining packed cells is stoppered with the lavender top and stored at room temperature with tubes #1 and #2. Tube #3 is shipped along with tubes #1 and #2 on the day of collection to the CBL for preparation of a stable cell lysate for DNA isolation.

Tube #4 remains incubating at room temperature for thirty minutes to allow the blood to clot. As soon as possible after the 30 minutes and not longer than 45 minutes after blood collection, tube #4 is centrifuged at 3000 rpm for 10 minutes at room temperature. After centrifugation, using a plastic transfer pipette, five equal aliquots of serum are transferred into

five microvials. These vials are capped and stored in the freezer until shipment to the CBL. The measurements performed at the CBL include serum lipids, creatinine, cystatin C and temporary storage at -70°C of three serum aliquots.

A1.2 Urine Specimen Collection

A random urine specimen is obtained at the clinical center during the patient's visit. The specimen is collected in a container labeled with the participant's GOKIND laboratory accession number. Collection time of the random specimen must be recorded on the Urine Mailing List.

Urine Aliquotting

All urine processing must take place behind a protective shield, either a desktop style shield or the type worn on the head. All other rules regarding safe blood specimen handling must be observed when processing urines.

The urine specimen container is inverted four times for thorough mixing of the specimen. Using a disposable pipette, approximately 4.5 mL of urine is transferred into each of four 4.5 mL storage tubes provided and appropriately labeled. All urine specimen tubes are placed in the freezer until shipment to the CBL. One urine sample tube is used for the albumin and creatinine assays at the CBL. Three urine specimen tubes are placed at -70°C for temporary storage at the CBL.

A1.3. Storage

The two CPT tubes and the one lavender top packed cells tube remain at room temperature before being shipped to the CBL on the day of collection. The one 0.5 mL whole blood microvial for hemoglobin A1c is placed into the refrigerator until shipment to the CBL within 5 days of collection. The one cryovial for lipids, the one cryovial for creatinine and cystatin C, the three serum aliquot tubes, the three plasma aliquot tubes, the one 4.5 mL aliquot tube for urine albumin and creatinine and the three 4.5 mL urine aliquot tubes are stored in the freezer before the weekly dry ice shipment to the CBL. .

A1.4. Packaging of Genetics Tubes (Tubes #1, #2, and #3)

Shipping must occur on the day of specimen collection. Specimen collection should be scheduled for Monday through Thursday (if possible), as tubes #1 and #2 and #3 must be shipped the same day for receipt at the Central Biochemistry Laboratory the following day (Tuesday through Friday, if possible).

Tubes #1, #2, and #3 remain in a rack at room temperature until shipment. Keep tubes #1 and #2 sealed to preserve sterility. Do not centrifuge tubes #1 and #2. Tube #3 is processed as described in Section 8.4.2 and the original lavender top tube with only the packed cells remaining is sent with tube #1 and #2. The tubes are shipped in a foam tube holder assembly placed inside a shipping box, both provided by the CBL. To package tubes for shipment:

1. Place tubes #1, #2 and #3 into the foam tube holder. Close the box containing the holder, and place it into an 8" x 10" ziplock bag. Seal the bag. If specimens are collected from more than one participant, additional foam tube holders can be used.

Multiple tube holder assemblies can be included in the same shipping box as described below.

2. Place a room temperature refrigerant pack on the bottom of the shipping box. Do not refrigerate or freeze the refrigerant pack. Put the foam tube holder assembly or multiply assemblies on top of the refrigerant pack.
3. Place packing material (crumpled paper, etc.) on top of the assemblies to occupy any remaining space in the box.
4. Place the Genetics Studies Mailing List for each participant specimen set included in the shipment on top of the packing material.
5. Seal the outer shipping box with strapping tape. Affix biohazard label to outside of box.
6. Address the shipping box using the preaddressed Federal Express air bill provided by the CBL. Place air bill into an air bill pouch affixed to outside of shipping box.
7. Contact Federal Express (800-GO-FEDEX) for pickup.

A1.5. Packaging of Refrigerated Hemoglobin A1c Specimens

The samples remain in the refrigerator until they are shipped. This must be accomplished within five days of collection.

The bags of refrigerated whole blood samples are packed and shipped with refrigerant packs in styrofoam boxes provided by the CBL. Packaging instructions are:

1. Place all 0.5 mL whole blood sample vials into a styrofoam 5-tube mailer (each slot can hold two vials) to protect from freezing. Place mailer into its cardboard sleeve.
2. Place the mailer into one 8” x 10” plastic storage bag.
3. Place a frozen refrigerant pack on the bottom of the styrofoam box.
4. Put the bag with mailer of sample tubes into the styrofoam box on top of the pack.
5. Place a second frozen refrigerant pack on top of the samples.
6. Place the Hemoglobin A1c Mailing List on top of the packing material.
7. Place the lid on the styrofoam box. Seal the outer box tightly with strapping tape. Affix biohazard label to outside of box.
8. Address box. Use the preaddressed Federal Express air bill provided by the CBL.
9. Contact Federal Express (800-GO-FEDEX) for pickup.

A1.6. Packaging of Frozen Specimens

Frozen specimens should be sent Monday through Thursday each week (being careful to avoid any weeks in which a holiday may occur). Shipping on Monday or Tuesday avoids problems in transporting the specimens over weekends. Any shipment deviations or questions should be discussed directly with the CBL. GOKIND Study samples can be sent with EDIC samples to the CBL.

Each clinical center should utilize the following protocol:

1. Place the frozen sample vials into plastic storage bags grouped by patient ID number and accession number assigned.

2. Using the insulated shipping containers provided by the CBL for frozen specimens, pack the specimens with at least two and a half to three pounds of dry ice. This amount should be increased during the warmer months.
3. Place a layer of dry ice on the bottom of the styrofoam transport box.
4. Place the 5-tube mailers on top of the dry ice.
5. Layer more dry ice and place remaining storage bags containing microvials on top of the dry ice. Place dry ice on top.
6. Enclose the completed Renal Studies, Lipid, Creatinine, Cystatin C and Saved Specimen Mailing Lists on top of the insulated shipping container prior to sealing the cardboard box. Affix biohazard label and dry ice label to outside of box.
7. Address box. Use the preaddressed Federal Express air bill provided by the CBL.
8. Contact Federal Express (800-GO-FEDEX) for pickup.

A1.7. Mailing Instructions to the CBL

All shipping containers are sent to the CBL by overnight Federal Express to ensure receipt within 24 hours. Shipping containers and other supplies will be returned to each of the clinical centers by UPS or the U. S. Postal Service. Shipping containers to the CBL are addressed as follows:

GOKIND Central Biochemistry Laboratory
Fairview-University Medical Center
Room L275 Mayo Memorial Building
420 Delaware Street S.E.
Minneapolis, MN 55455
Telephone: (612) 273-3391 (office)
Telephone: (612) 273-3645 (lab)

Appendix 2: Consent Form

NAME OF CENTER

Committee on Human Studies

Informed Consent Form

Study Participant's Name: _____

Principal Investigator:

Co-Investigator(s):

Project Title: *Genetics of Kidneys in Diabetes (GoKinD) Study*

Part To Be Read and Signed by Participant:

We want you to understand the risks and benefits of this research study before agreeing to participate. This is known as "informed consent." This study is being conducted by the Joslin Diabetes Center, George Washington University, EDIC (Epidemiology of Diabetes Interventions and Complications) associated Centers, participating renal care centers, the University of Minnesota, and the Centers for Disease Control and Prevention (CDC), and is sponsored by the Juvenile Diabetes Research Foundation, International (JDRF). Local recruitment site: _____.

This informed consent form may contain words that you do not understand. You may ask the study investigator or a staff member to explain any words or information.

PURPOSE OF STUDY

We are asking you to let us have samples of your blood and urine to store for future research on the genetic risk factors for type 1 diabetes and its complications. We will share these samples and study information with many investigators studying the role that genes play in causing these diseases.

PARTICIPANT SELECTION

We are asking you to be in this study because you have Type 1 Diabetes or you have a child with Type 1 Diabetes. Around 4,300 individuals will participate.

Your participation is voluntary. You may decline to participate or you may leave the study at any later time. Either way, you will not be penalized or lose any benefits to which you are otherwise entitled.

ABOUT THE STUDY

We will clean an area on your forearm with alcohol and insert a needle into a vein. Then we will draw a sample of blood (1 to 2 ounces) into a tube. We will use the blood to check your kidney function, blood proteins, and blood cells. You should not participate in this study if you have donated blood within the past two weeks. Also, you should not donate blood for two weeks after we draw blood. You will need to give us a urine sample. We will check it for protein and infection. We will measure your height, weight, and blood pressure. You'll be asked to fill out questionnaires about your medical history, your diet, and your diabetes. We may ask for your permission to obtain medical information from a doctor that you have seen. The information you give us, plus the blood and urine results, will go to The George Washington University Biostatistics Center in Washington, D.C. They will be collecting information from study participants at other medical centers as well.

We may contact you in the future by letter or telephone to find out how you are or to see if you would like to participate in another study.

We will use part of your blood sample to collect DNA. We will use another part of the blood sample to grow your white blood cells in the laboratory. The CDC in Atlanta, Georgia will be responsible for storing your DNA and properly caring for your cells.

Researchers will study the DNA and other parts of your white blood cells for genes that make people more or less likely to get diabetes and its complications. At the present time, we do not know all of the ways that information from DNA and white blood cells may help us. That is why we will store them. The CDC will collect more DNA from your stored white blood cells as needed.

The researchers who study the DNA must meet the scientific standards of the Juvenile Diabetes Research Foundation, International (JDRF), the sponsor of this study. To further qualify, the researchers must also be from a facility approved by the Office for Human Research Protections located in the Office of the Secretary, Department of Health and Human Services.

The CDC will give your DNA or other parts of your cells to approved researchers. The CDC will not give your cells to the researchers or anyone else. The George Washington University Biostatistics Center will give your study information to the same researchers. Once the researchers receive your DNA and other parts of your cells, neither the CDC nor the JDRF can guarantee how they will be used.

The DNA and cells will be stored indefinitely unless you request that they be destroyed. Your continued participation, however, is voluntary. You may leave the study at any time without penalty or losing any benefits to which you are otherwise entitled. If you decide that you no longer want your DNA, white blood cells, blood, or urine used for this study, you may request in writing that all remaining samples be destroyed. Your request will be carried out. We cannot retrieve DNA samples that have already been sent out or information that has been sent out. Requests should be sent to:

[Enter Site Specific Contact Information]

At times findings or inventions from samples in research studies may have value if they result in products that can be made or sold. If this should happen you would not share in any money made from these products.

RISKS AND/OR DISCOMFORT

You may feel a sharp sting from the needle used to draw your blood. Sometimes, a bruise or small blood clot appears at the site. These usually disappear on their own or with applied heat. Although it rarely happens, the needle could cause damage to a nerve. This can cause numbness in part of the arm.

We will take steps to keep information that could identify you separate from coded medical information (see paragraph marked CONFIDENTIALITY). However, you should be aware that there is always a risk of medical information getting out. For example, you may accidentally learn that you have a high risk of a disease besides diabetes. A court of law might order the investigators at Joslin Diabetes Center to release your medical information. Members of your family or others may learn information about you. You may discover that a relative is not related by blood. For example, an adoption that has been kept a secret may become known.

Medical information has been used to deny people employment, insurance and housing.

If you have any questions or think you have been injured in this study, you should immediately contact the study investigator, [Enter Site Specific Name].

BENEFITS

There is no guarantee that you will benefit from this study. Your participation may help us discover ways to prevent diabetes and its complications.

The results of standard medical tests will be given to you. Study results from your DNA or white blood cells will not.

ALTERNATIVE PROCEDURES

You may choose not to be in this study.

COST/PAYMENT

There will be no charge to you for participating in the study. You will be paid $50.00 as reimbursement for your inconvenience. Parking will be provided free of charge.

CONFIDENTIALITY

Your medical information will be protected by the confidential information policies of Joslin Diabetes Center and The George Washington University Biostatistics Center in Washington, D.C. No information that could identify you will be released. However, there is always a risk that this information may become known. For example, it is conceivable that medical

information could get out during electronic transmission of your data. To reduce the risk, medical records will be kept in restricted areas at [Local site]. Computer access will be limited to authorized staff members of the [Local site] and the Biostatistics Center. Information that could identify you, such as your name, will be kept in a file apart from medical information.

A code number will be used on your medical information. Investigators who look at your medical information will not be able to identify you. Only the Biostatistics Center will know to whom the code number belongs.

Results of this study may be published in scientific journals or presented at medical meetings. You will not be personally identified.

To further protect the privacy of your medical records, do not publicly state any information about your family's medical history or your participation in this study.

SIGNATURE PAGE

I am informed about and understand the purpose of the above-described research study and its procedures. The possible discomfort, risks, and benefits were explained. This includes foreseeable and unforeseeable effects. I should not expect free medical care or payment if I'm injured in this study. I will discuss the study with my primary care physician.

I understand that the study may be published or shared for scientific purposes. My name will not be used and every effort will be made to protect my privacy. I don't expect to profit if this study results in development of any marketable product.

If I have questions about my rights as a study participant, I should contact the Committee on Human Studies of the [Local site]. I can call The Committee's Chairman or Coordinator. They may also contact me or look at my study records.

[Enter Site Specific Name]	**[Enter Site Specific Name]**
IRB Coordinator	**Chairman, CHS**
Telephone:	**Telephone:**

I understand that I may leave the research study or refuse any procedure at any time without prejudice to me.

I voluntarily consent to participate in this research study and have received a copy of this form.

Signature of Participant or Participant's Representative	*Date*	*Relationship to Participant (parent, sibling, guardian)*

Part to be completed by Investigator (or Representative)

I have explained to the above-named participant the nature and purpose of the research study outlined above. This includes foreseeable discomfort, risks, and benefits. I have considered and rejected alternative procedures for obtaining this information. I have offered to answer any questions the participant may have about the study and have answered these questions to the best of my ability.

Signature of Investigator (or Representative)	*Date*

Appendix 3: Evaluation of criteria for defining absence of nephropathy

Definition of Absence of Nephropathy from Protocol Section B.1:

"Subject has persistent normoalbuminuria despite duration of type 1 diabetes for at least 15 years and has never been treated with ACE inhibitors.

"Persistent normoalbuminuria is defined as at least 2 out of 3 ACR measurements (a least 1 month apart) in random urine specimens being less than 20 μg of albumin/mg of creatinine. If 3 ACR measurements are needed, the highest must also be less than 40 μg of albumin/mg of creatinine."

Natural History of Microalbuminuria Study The charge to the Coordinating Center was to examine the performance of these criteria using the data accumulated by the "Natural History of Microalbuminuria Study," which has been in progress at the Joslin Diabetes Center since 1991. For that study we screened a 50% sample of Joslin Clinic patients aged 15-44 years for microalbuminuria using the ACR determined in multiple, random urine samples. Since then, we have followed roughly 800 patients with type 1 diabetes and normoalbuminuria to detect the onset of microalbuminuria. We followed an additional 314 patients with type 1 diabetes and microalbuminuria to detect progression to proteinuria. Microalbuminuria in this group was defined as persistent ACRs in the range 17 to 249 μg of albumin/mg of creatinine for men and 25 to 354 μg of albumin/mg of creatinine for women (equivalent to a urinary albumin excretion rate in the range 30 to 299 μg of albumin/min). Proteinuria was defined as an ACR above these ranges.

Onset of Microalbuminuria within 4 Years Follow-up

Protocol criteria: From the study group of patients with normoalbuminuria, we selected those with 15 years duration of diabetes in 1991 whose classification was based on 3 ACRs (at least 8 weeks apart), 2 of which were < 20 μg of albumin/mg of creatinine and none was as high as 40 μg of albumin/mg of creatinine. None were treated ACE inhibition. Average duration was 22 years.

N = 202 Microalbuminuria developed in 24 (12%) during 4 years follow-up.

More stringent ACR criterion: We made the ACR criterion more stringent: 2 out of 3 < 15 μg of albumin/mg of creatinine and none as high as 40 μg of albumin/mg of creatinine.

N = 198 Microalbuminuria developed in 22 (11%) during 4 years follow-up.

If we increased further the stringency of the ACR criterion, we simply reduced the number of qualifying patients without reducing the incidence of microalbuminuria.

More stringent diabetes duration criterion: We increased the minimum duration of diabetes to 20 years.

N = 123 Microalbuminuria developed in 13 (11%) during 4 years follow-up.

This reduced the number of patients by almost a half, but did not reduce the incidence of microalbuminuria.

Conclusion: In terms of progression from normoalbuminuria to the stage of microalbuminuria, we cannot improve the performance of our screening criteria by increasing the stringency of either criterion (ACR level or diabetes duration).

Progression of Microalbuminuria to Proteinuria within 4 Years Follow-up

The presence of microalbuminuria does not mean that proteinuria is inevitable. To get an idea of the fate of these patients who develop microalbuminuria, we examined the study group of patients with microalbuminuria being followed at the Joslin. From that study group, we selected those with 15 years duration of diabetes at baseline who were not treated with ACE inhibitors. The average duration in this groups was also 22 years.

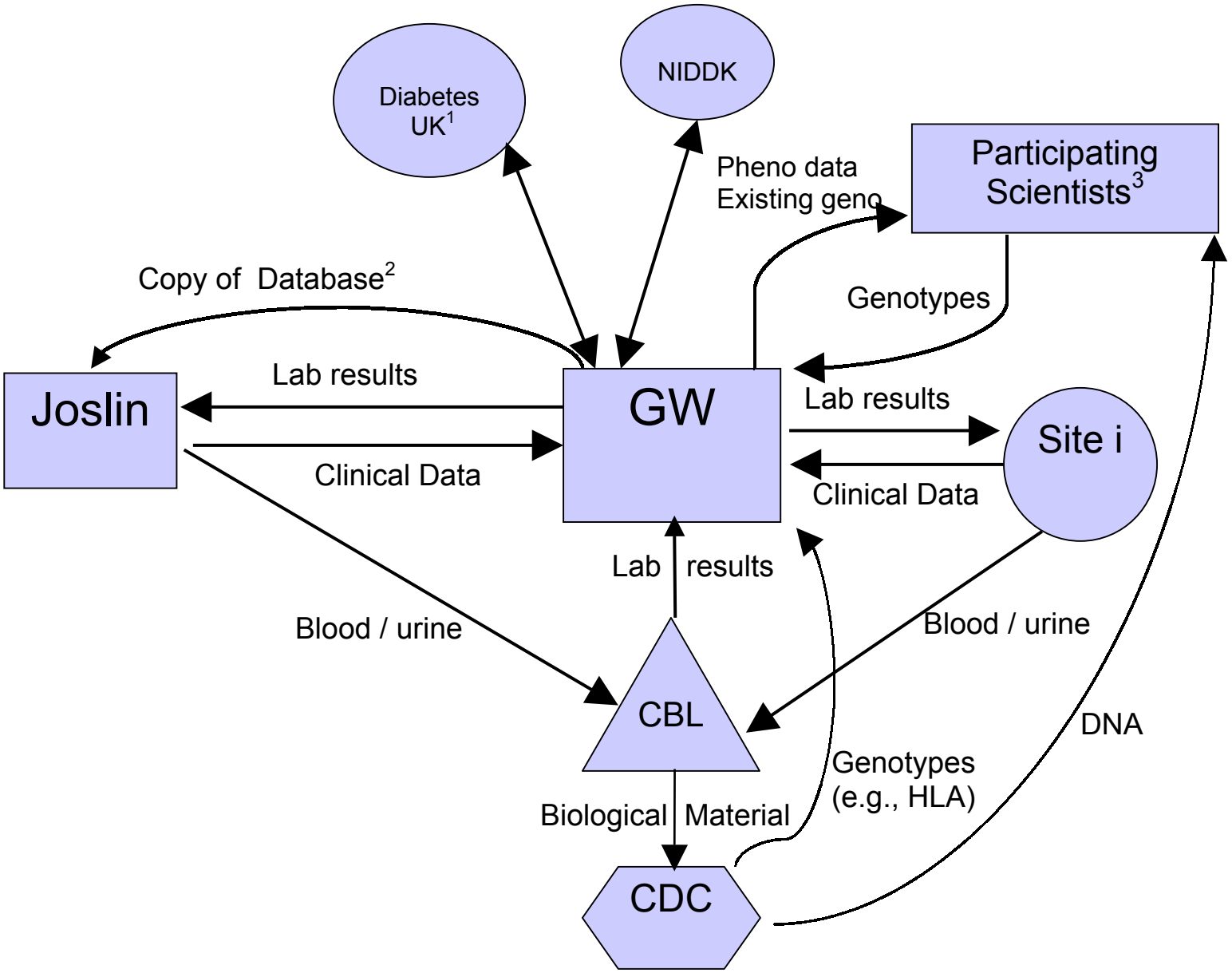
N = 127 Proteinuria developed in 16 (13%) during 4 years follow-up,
Microalbuminuria persisted in 70 (55%)
Normoalbuminuria developed in 41 (32%)

Using our **current protocol criteria**, and the Joslin data on the natural history of microalbuminuria, we can estimate that out of 500 subjects with normoalbuminuria, 59 will develop microalbuminuria during 4 years follow-up. Of these 59 new cases of microalbuminuria, 7 or 8 will develop proteinuria within another 4 years of follow-up while 19 will regress to normoalbuminuria and the remainder will remain in the microalbuminuria stage.

Extrapolation of Results to 12 Years Follow-up: Assume constancy of transition rates over time (the incidence rate of microalbuminuria, the incidence of proteinuria among patients with microalbuminuria, and the rate of regression from microalbuminuria to normoalbuminuria). In 2015, the 500 subjects with normoalbuminuria selected in 2000-2003 will have 34 years duration of diabetes (on average) and will be distributed as follows.

Normoalbuminuria:	389	(77.8%)
Microalbuminuria:	93	(18.6%)
Proteinuria	18	(3.6%)

Figure 1: Organizational Diagram for the GoKinD study



[1] Plus other international sites
[2] Backup copy will also be sent to CDC
[3] Applications from Joslin and George Washington University will receive expedited review

3/6/01