1.0 INTRODUCTION

The Family Investigation of Nephropathy and Diabetes (FIND) is designed to identify genes responsible for diabetic nephropathy and their linkage relationships, if any, to nephropathy. The goal of this study of diabetic retinopathy in this population is to evaluate whether there is a genetic link between diabetic nephropathy and diabetic retinopathy**.** Are there genetic factors that predispose to both diabetic nephropathy and retinopathy? Are there genetic factors that predispose uniquely to either retinopathy or nephropathy respectively? A secondary goal is to perform a genetic linkage study of more severe diabetic retinopathy.

The initiation and progression of diabetic retinopathy and nephropathy are both impacted by glycemic control[1]. However, the risk factors for retinopathy and nephropathy are not identical. For instance, increasing duration of diabetes is a risk factor for retinopathy[2], but not necessarily for nephropathy, where the risk for the development of renal disease after more than two decades of diabetes may slowly decline, particularly for Type 1 diabetes[3] but probably also for Type 2.

Genetic factors may contribute to the initiation and/or rate of progression of diabetic retinopathy. In support of a genetic hypothesis, some studies[4,5] but not all[6] suggest that Hispanic subjects with diabetes develop more severe retinopathy earlier and progress more rapidly than African-Americans or European-Americans independent of glycemic control or other environmental considerations. One study suggested that retinopathy was also less prevalent in African-Americans than in European-Americans[7]. In one study involving Finnish adolescents with IDDM, expression of the HLA DR1 locus has been suggested as potentially predisposing to diabetic retinopathy whereas HLA A9 and B40 may have had slight protective effects[8]. In another study, involving Pima Indians with NIDDM, linkage to both nephropathy and retinopathy was found on chromosomes 3 and 9[9]. A study in NIDDM subjects from India suggested an association between retinopathy and the G2m locus encoding the IgG2 subclass heavy chains[10]. Results from the Diabetes Control and Complications Trial (DCCT) study of familial aggregation showed that the severity of diabetic retinopathy is influenced by familial (possibly genetic) factors and confirmed the effect of familial factors in the development of diabetic nephropathy.[11]

2.0 FIND

The NIDDK-sponsored FIND will recruit probands with diabetic nephropathy and their family members. The majority of the probands will be affected with diabetic retinopathy of various degrees of severity. FIND is a multi-center consortium, comprising eleven (11) Participating Investigator Centers (PICs) and a Genetic Analysis and Data Coordinating Center (GADCC), established to study the genetics of Diabetic Nephropathy. Seven (7) of the PICs, a central fundus photograph reading center, and the GADCC are participating in the FIND Retinopathy Study, which is being sponsored by the National Eye Institute (NEI).

The charge of the consortium is to acquire sets of families with well-characterized diabetic nephropathy, establish a secure master FIND database, and perform a genome scan to identify chromosomal regions linked with diabetic nephropathy. Analytic methods will include a) affected sibling pair (ASP), discordant sibling pair (DSP), affected relative pair (ARP), and discordant relative pair (DRP) linkage analyses and b) Mapping by Admixture Linkage Disequilibrium (MALD) analyses.[12,13,14,15,16] For group a, the genetic analysis will consist of candidate gene and linkage analysis. Only the probands recruited into group a, the affected sibling pair/discordant sibling pair and affected relative pair/discordant relative pair will participate in the retinopathy study. If a proband is unable to participate in the retinopathy study, family members may still be enrolled as long as at least two diabetic siblings enroll. The seven PICs participating in the retinopathy study and the anticipated number of probands to be enrolled from each are listed below:

Case-Western Cleveland	180
Wake Forest U.	310
Harbor UCLA	80
U. of New Mexico	126
U. of Texas, San Antonio	180
University of California, Los Angeles (UCLA)	270
NIDDK Phoenix	148

There will be a total of 1,294 probands in the entire study of diabetic retinopathy. We expect to find 1.5 siblings/relatives who are also diabetic per proband. The total number of participants requiring eye examinations and fundus photographs will be 3,235. These participants will be recruited over a period of 3.5 years. This will be a cross-sectional study. No follow-up information will be collected.

2.1 Standard Procedure for Eye Examinations and Fundus Photographs

Probands and family members who are diabetic will undergo an eye examination and fundus photography. Family members entered into the genetic database who are not diabetic, such as non-diabetic parents, will not undergo eye examination or fundus photography. For participants examined at the PICs, seven stereoscopic fields will be obtained by photographers who are certified by the central fundus photographic reading center. The reading center will grade the fundus photographs using the modified Early Treatment Diabetic Retinopathy Study (ETDRS) classification of diabetic retinopathy.[17,18] The study ophthalmologist from each of the PICs will see the majority of the participants, both the probands and the family members with diabetes. However, for those participants who will not be able to travel to the PICs, the reading center will assist in identifying a collaborating ophthalmologist who will perform the eye examination and obtain fundus photographs, using the 7-field or a simplified photographic procedure. The ophthalmologist will be asked to fill out an eye examination form, which will be sent with the photographs to the reading center. A part-time clinical coordinator will be funded at each clinical center to ensure that the eye data (data forms and fundus photographs) are collected and transmitted to the reading center in a timely fashion for

all eligible participants.

2.2 Alternative Procedure for Obtaining Information from Participants' Eye Care Provider

All efforts should be made to enter participants into the full Eye protocol, including obtaining fundus photographs and eye examinations. However, for those participants who have expired, or for those who are unwilling or unable to schedule a FIND eye exam but are willing to permit information to be obtained from their eye care providers, the following should be done:

Make sure that the PIC has a copy of a valid consent form or medical records release to send to the eye care provider to obtain information from medical records.

Send the eye care provider a blank FIND Eye Exam Form for documentation of the eye exam. Ask the eye care provider to review the patient's record and to fill out the FIND Eye Exam Form based on the most recent visit, also using additional information from previous visits as needed. The forms should be returned to the reading center. These data will be used to create retinopathy severity scores in the absence of fundus photographs. See appendices 7-9.

2.3 Procedures for Obtaining Fundus Photograph Data from the NIDDK Phoenix PIC

FIND Eye Study data from the NIDDK Phoenix PIC 10 is being obtained from data collected for another research study, rather than from scheduled appointments for FIND eye exams and fundus photographs. See appendix 10.

3.0 STATISTICAL ANALYSES

All analyses will include ethnicity as a covariate, as appropriate. Initial analysis will be to estimate, for each of the ethnicities and each of the quantitative traits measured, the sibling correlation in this sample. To do this we will use the S.A.G.E. (2001) program FCOR2 that estimates the multivariate correlations and their standard errors without assuming multivariate normality. Only those traits for which sibling correlations are significant at the 5% level will be considered further. Single-point and multipoint model-free linkage analysis will be performed on these quantitative traits, starting with the traits with highest sibling correlations. First we shall use the most powerful version of the Haseman-Elston (2000) regression method available at the time, as implemented in the S.A.G.E. (2001) programs GENIBD and SIBPAL2. This will include multivariate analysis, which is currently being developed. For those traits that show strong linkage signals, model-based linkage analysis will also be performed after fitting an appropriate segregation model (it is anticipated that such a model will necessarily include familial correlations due to causes other than segregation at the locus to be linked).

Based on these analyses, denser mapping will be undertaken of implicated chromosomal regions and the linkage analyses repeated.

Retinopathy severity will also be considered in some analyses using the classification described below, which is based on the more severely involved eye.

1) Very mild or no retinopathy:

   Retinopathy severity level 10 (no retinopathy) or 20 (aneurysms only) and date of examination/photographs >= 15 years after date of diagnosis of diabetes.

2) Retinopathy of intermediate severity, defined as either of the following:

   a) Retinopathy severity as defined in 1) above, but date of examination/photographs < 15 years after date of diagnosis of diabetes;
   b) Retinopathy more severe than 1) above but not as severe as 3) below.

3) Severe retinopathy, defined as any one or more of the following:

   a) Retinopathy severity level 53 (severe nonproliferative retinopathy) or greater (proliferative retinopathy),
   b) Clinically significant macular edema,
   c) Presence of scars of scatter (panretinal) photocoagulation in fundus photographs,
   d) Presence of definite scars of focal/grid photocoagulation for macular edema in fundus photographs or questionable presence of such scars and a history of photocoagulation.

Depending on the results of all these analyses, joint analyses of retinopathy and nephropathy may be performed, and putative candidate genes may be investigated by methods of association (TDT) or association/linkage analysis.

3.1 Power Considerations

An indication of the minimum power that will be available can be inferred from the calculations performed by Amos et al (1989) and the theory developed by Blackwelder and Elston (1982) for a less powerful univariate model-free linkage test. Quantitative traits based on fundus photography on a total sample of 1350 diabetic probands and 2025 of their diabetic siblings will be available. Assume (conservatively) that these will comprise 675 sib pairs and 675 sib trios. Each trio gives rise to 3 x 675 = 2025 sib pairs, so that we can assume a total sample of 675 + 2025 = 2700 sib pairs. Assuming fully informative markers (as will be approximated by performing linkage analysis in a densely mapped region), Amos et al's (1989) results suggest that 2700 sib pairs should be sufficient to detect a locus at which an additive or dominant allele is segregating and accounts for about 30% of the variance of the trait in this sample (sample locus-specific

heritability = 0.3, correspond to a sibling correlation of 0.15 under simplifying assumptions) provided the allele frequency lies between 0.1 and 0.5. The results they actually present indicate that 1200-1300 pairs will detect such a linkage at the 5% level with 95% power. From this we can project that a sample twice this size will detect linkage at the 2×10^{-4} significance level with 80% power, using the theoretical results given by Blackwelder and Elston (1982). A significance level of 1×10^{-4} is usually used when performing a genome-wide scan; the newer methods of analysis can be expected to attain this level of significance under the conditions indicated.

4.0 EYE EXAMINATION PROCEDURES

4.1 Introduction

The procedures for carrying out the eye examinations required in the study are described in this section. Required ocular examinations include visual acuity measurement, intraocular pressure measurement, and ophthalmoscopic examination.

The procedures to be used in the PICs for taking fundus photographs and transmitting them to the reading center are described in appendices 1, 2, and 4. Comparable procedures for examinations and fundus photographs carried out in the offices of collaborating ophthalmologists are described in appendices 3-5.

4.2 Brief History

The patient's birth date, year of diabetes diagnosis, and history of insulin use and of prior ocular treatments are recorded (see Eye Exam Form).

4.3 Visual Acuity Measurement

A staff member in the examining ophthalmologist's office should conduct the visual acuity measurement with the method customarily used in that office using the patient's glasses, if available. If visual acuity is worse than 20/40, a pinhole should be added.

4.4 Intraocular Pressure

Intraocular pressure (IOP) should be measured using an applanation tonometer by personnel experienced in the procedure. A pneumatonometer may be used if an applanation tonometer is not available.

4.5 Pupil Dilation and Fundus Photography

Photographs should be taken through a maximally dilated pupil. It is recommended that 2 sets each of 2.5% Neo-synephrine and 1% Mydriacyl be instilled 2-5 minutes apart. Photographs should be taken prior to any planned contact lens examination, which may distort the tear film and impair the quality of photographs. See Appendices 1 and 2 for the fundus photography procedures for the PICs and Appendix 3 for the fundus photography procedures for collaborating ophthalmologists.

4.6 Ophthalmoscopic Examination

The ophthalmologist may use his or her usual examining technique, which should include direct ophthalmoscopy or slit-lamp biomicroscopy with precorneal or contact lens in order to provide adequate magnification for detection of microaneurysms.

The following items should be recorded (see Appendix 4 for the Eye Exam Form):

- Retinopathy severity level;
- Presence or absence of scars of panretinal photocoagulation (or local photocoagulation, presumably for new vessels);
- Presence or absence of scars of focal or grid photocoagulation for macular edema;
- Presence or absence of macular edema (retinal thickening, with or without lipid deposits, within one disc diameter of the center of the macula), and, if present, whether or not the center of the macula is involved;
- If visual acuity is worse than 20/40 (with pinhole, if used), primary and contributing causes of the decreased acuity.

4.7 Appointments with Collaborating Ophthalmologists for Family Members

The central fundus photograph reading center will compile a list of collaborating ophthalmologists throughout the United States and Puerto Rico. If the proband's sibling /relative lives outside the PIC area, the PIC will use this list to locate a nearby ophthalmologist to schedule the eye examination and photography. Upon notification of the scheduled appointment, the reading center will send the collaborating ophthalmologist a packet of information and instructions (see Appendices 3, 4, and 5). The reading center needs to be notified at least 2 weeks prior to the scheduled appointment to insure that the packet is received prior to the appointment. The ophthalmologist will return the Transmittal Log, Eye Exam Form, and Invoice with the photographs to the reading center, who will communicate receipt of the information to the PIC.

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