

Heritability of the Severity of Diabetic Retinopathy: The FIND-Eye Study

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PURPOSE. Diabetic retinopathy (DR) and diabetic nephropathy (DN) are serious microvascular complications of diabetes mellitus. Correlations between severity of DR and DN and computed heritability estimates for DR were determined in a large, multiethnic sample of diabetic families. The hypothesis was that (1) the severity of DR correlates with the presence and severity of nephropathy in individuals with diabetes mellitus,

and (2) the severity of DR is under significant familial influence in members of multiplex diabetic families.

METHODS. The Family Investigation of Nephropathy and Diabetes (FIND) was designed to evaluate the genetic basis of DN in American Indians, European Americans, African Americans, and Mexican Americans. FIND enrolled probands with advanced DN, along with their diabetic siblings who were concordant and discordant for nephropathy. These diabetic family members were invited to participate in the FIND-Eye study to determine whether inherited factors underlie susceptibility to DR and its severity. FIND-Eye participants underwent eye examinations and had fundus photographs taken. The severity of DR was graded by using the Early Treatment Diabetic Retinopathy Study Classification (ETDRS). Sib-sib correlations were calculated with the SAGE 5.0 program FCOR, to estimate heritability of retinopathy severity.

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RESULTS. This report summarizes the results for the first 2368 diabetic subjects from 767 families enrolled in FIND-Eye; nearly 50% were Mexican American, the largest single ethnicity within FIND. The overall prevalence of DR was high; 33.4% had proliferative DR; 7.5%, 22.8%, and 9.5% had severe, moderate, and mild nonproliferative DR, respectively; 26.6% had no DR. The severity of DR was significantly associated with severity of DN, both by phenotypic category and by increasing serum creatinine concentration ($\chi^2 = 658.14$, $df = 20$; $P < 0.0001$). The sib-sib correlation for DR severity was 0.1358 in the total sample and 0.1224 when limited to the Mexican-American sample. Broad sense heritabilities for DR were 27% overall and 24% in Mexican-American families. The polygenic heritability of liability for proliferative DR approximated 25% in this FIND-Eye sample.

CONCLUSIONS. These data confirm that the severity of DR parallels the presence and severity of nephropathy in individuals with diabetes mellitus. The severity of DR in members of multiplex diabetic families appears to have a significant familial connection. (*Invest Ophthalmol Vis Sci.* 2008;49:3839-3845) DOI:10.1167/iovs.07-1633

Diabetic retinopathy (DR) and diabetic nephropathy (DN) are serious microvascular complications of type 1 and 2 diabetes mellitus (DM).¹ DR is a leading cause of blindness among adults in the United States, affecting more than 60% of individuals with type 2 DM.² Approximately 4.1 million American adults have DR, and one of every 12 diabetic persons older than 40 years has vision-threatening retinopathy.³ The development and progression of DR is associated with the duration of diabetes in both type 1 and 2 diabetic patients.⁴ Nearly all

patients with type 1 diabetes and 60% of patients with type 2 diabetes have retinopathy during the first two decades of disease. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), 3.6% of younger-onset patients (type 1 diabetes) and 1.6% of older-onset patients (type 2 diabetes) were legally blind. In the younger-onset group, 86% of blindness was attributable to DR. In the older-onset group, in which other eye diseases were common, one-third of the cases of legal blindness were due to DR.^{4,5}

DR is characterized by microvascular abnormalities, proliferation of retinal vessels and increased retinal vascular permeability, leading to the development of nonproliferative DR (NPDR), proliferative DR (PDR), and macular edema (DME), respectively.⁶ Vision loss in DR can result from several mechanisms. Central vision may be impaired by macular edema or capillary nonperfusion. New blood vessels of PDR and contraction of the accompanying fibrous tissue can distort the retina and lead to tractional retinal detachment, producing severe and often irreversible vision loss. In addition, the new blood vessels may bleed, adding further complications of preretinal or vitreous hemorrhage.⁴

Currently, the gold standard for determining severity of DR is the grading system that has been modified and tested by the Early Treatment Diabetic Retinopathy Study (ETDRS), on seven standard field 35-mm stereoscopic color fundus photographs.⁷ This system takes into consideration the severity of the abnormalities characterizing both nonproliferative and proliferative retinopathy, including neovascularization, fibrous proliferation, vitreous and preretinal hemorrhage, and scars of panretinal photocoagulation.

The presence of DR, as well as the type of retinal lesions, aggregates in families.⁸⁻¹⁰ It has been suggested that Hispanic individuals with diabetes develop severe retinopathy earlier in their course and progress more rapidly compared with African Americans or European Americans.^{11,12} These observations were independent of glycemic control and measured environmental factors. A lower prevalence of DR has also been reported in diabetic African Americans compared with that in European Americans.⁹ In the families in the Diabetes Control and Complications Trial (DCCT), the risk of severe DR was higher among relatives of subjects with severe DR than in relatives of those with mild or moderate DR.¹³ In South-Indian families containing multiple type 2 diabetic siblings, the prevalence of DR was three times higher in siblings of probands with DR than in those without.¹⁴

DN is a microvascular complication of diabetes that is characterized by persistent albuminuria and secondarily elevated blood pressure, followed by a progressive decline in kidney function. It is the most common cause of end-stage renal disease (ESRD) worldwide and accounts for 40% of incident dialysis patients in the United States.¹⁵ DN clusters in families with both type 1 and 2 diabetes, suggesting a genetic component to the development and/or progression of disease.¹⁶⁻²¹

DR and DN frequently coexist; DR is more severe in patients with severe DN and those with advanced DN have far more severe lesions of DR than do those without DN.^{1,8} The rate of renal disease progression among proteinuric patients with type 2 DM is reportedly faster in those with concomitant retinopathy, after adjustment for blood pressure, metabolic, and lipid control.²² Relatively few existing data sets have included factors influencing correlations between DR and DN in multiplex diabetic families or across different ethnic groups. The FIND study was initiated to map genes influencing DN susceptibility. FIND enrolled families with probands who had advanced DN, along with their diabetic siblings, concordant and discordant for nephropathy. All FIND family study members were invited to participate in the FIND-Eye substudy in an attempt to localize genes underlying susceptibility to DR. Our goal was to

assess the following hypotheses: (1) Is the severity of DR associated with the presence and severity of nephropathy in individuals with diabetes mellitus, and (2) is the severity of DR under significant familial influence in members of multiplex diabetic families?

METHODS

Enrollment of Family Members in the FIND-Eye Study

Family members enrolled in the FIND and FIND-eye studies were self-reported European Americans, African Americans, Mexican Americans, and American Indians. Families eligible to enroll in FIND-Eye contained a proband with advanced DN and at least one other diabetic sibling, regardless of DR status. Eligible subjects underwent eye examinations and 30° stereoscopic fundus photography of seven fields. Grading of the fundus photographs and/or eye examination information was conducted centrally, in a masked fashion, by the Fundus Photograph Reading Center (FPRC) at the University of Wisconsin.

The FIND-Eye is predominantly a sib pair study (>90% of families); however, living parents and other relatives (i.e., avuncular, cousin, half-sibling, and grandparental affected pairs) were recruited when available. Sibling status was determined based on subjects' self-reported responses to questions presented on the FIND screening questionnaire. These questions were formulated to collect data related to family structure and kinship relationships. This study was approved by the Institutional Review Board at each participating institution, including the data-coordinating center. An informed consent was obtained from all participants. The protocol complied with the tenets of the Declaration of Helsinki, and a certificate of confidentiality was filed at the National Institutes of Health.

Phenotype Descriptions

Definitions and measurements of DM and DN phenotypes for subjects enrolled in the FIND-Eye substudy were based on definitions in the parent FIND, previously described in Knowler et al.²³ Most of the enrolled subjects had type 2 diabetes, based on disease onset after the age of 30, and at least 6 months of treatment with diet and/or oral agents. In addition, there are no established methods to differentiate between type 1 and 2 diabetes in patients with renal insufficiency or end-stage renal disease. Because a large proportion of our probands were so affected, antibody studies and C-peptide levels were not a part of our protocol.

Diabetes was clinically diagnosed in subjects currently treated with insulin and/or oral hypoglycemic medications. Subjects reporting DM and not undergoing active medication treatment had HbA1c measured at study entry. HbA1c concentrations above 6.0% were suggestive of DM and follow-up with the participant's physician was suggested. Confirmation of a diabetes diagnosis in these individuals qualified the participant for the study. American Diabetes Association²⁴ criteria were used for participants not previously known to have DM. In previously diagnosed individuals, the date of diabetes diagnosis was obtained from a medical questionnaire, with confirmatory medical record review where possible. For more information about diabetes definitions, please see Knowler et al.²³

The proband in FIND families had advanced DN. In the FIND-Eye, family entry required recruitment of at least one additional informative diabetic sibling. DN was diagnosed with (1) a kidney biopsy revealing DN with overt proteinuria (≥ 0.5 g protein/g creatinine); (2) ESRD attributed to DN by onset of DM ≥ 5 years before renal replacement therapy and documented DR (microaneurysms, PDR, macular edema, or prior retinal laser photocoagulation) and onset of DM ≥ 5 years before renal replacement therapy with proteinuria (≥ 3.0 g protein/g creatinine) or DR with proteinuria (≥ 3.0 g protein/g creatinine); or (3) chronic kidney disease due to DN based on DR with overt proteinuria (≥ 1.0 g protein/g creatinine) or DM duration of ≥ 10 years with heavy proteinuria (≥ 3.0 g protein/g creatinine). If the proband had had

known diabetes for >10 years, DR was not required for eligibility. If the proband had had diabetes for 5 to <10 years, at least nonproliferative DR was an entry requirement.

The majority ($n = 1854$, 78.3%) of FIND-Eye participants underwent a new eye examination and stereoscopic fundus photography of seven fields in both eyes with the ETDRS protocol.⁷ Retinal photographs were graded in a standardized manner, and DR severity was classified according to the ETDRS DR severity system.²⁵ Twelve percent ($n = 288$) were not photographed but had useful information obtained from prior eye examinations that were scored at the FPRC at the University of Wisconsin. An additional 10% ($n = 226$) had fundus photography previously performed and graded by using similar procedures.⁷ Eye data for these 226 subjects were collected from an ongoing longitudinal study among the Pima Indians conducted at NIDDK-Phoenix. All participants at the Phoenix site had retinal photographs taken through dilated pupils (two standard 45° fields for each eye) with a fundus camera (model CR4-45NM; Canon, Tokyo, Japan) centered on the optic disc and at the macula.²⁶ In all data sets, the severity of DR was classified according to the ETDRS system.⁷

A numeric score was assigned by the FPRC at the University of Wisconsin. Readers had no knowledge of the clinical status of FIND-Eye participants. Scores classified DR as follows: no DR (ETDRS = 10–12), mild NPDR (ETDRS = 14–20), moderate NPDR (ETDRS = 35–43), severe NPDR (ETDRS = 47–53), and proliferative DR (ETDRS ≥ 60). Accordingly, the following categories were created, based on the more severely involved eye: (1) no DR, (2) mild NPDR, (3) moderate NPDR, (4) severe NPDR, and (5) PDR. These categories were rescaled to estimate familial correlations for DR, and the Mendel and Elston²⁷ approach was used to estimate the polygenic heritability of liability for PDR. When semiquantitative scales are developed to grade disease, each step along the scale is not always equivalent (i.e., going from step 1 to 2 may not be equivalent to going from step 2 to 3). Factors contributing to difficulties in interpretation of the different parts of the scale are interactions between covariates. The goal of the rescaling was to minimize interactions with other variables. We used the rescaled scores to determine heritability and studied the relationship between the rescaled score and covariates including age, sex, ethnicity, body mass index, diabetes duration, serum creatinine concentration, and urine ACR.²³

Statistical Analyses

Estimates of heritability (b^2) for DR and liability for PDR were derived from sib-sib correlations. To estimate the familial correlation of DR, we used the method of Fisher²⁸ to rescale the original retinopathy severity scores (1, 2, 3, 4, and 5) in such a way that the proportion of the sum of squares due to interactions between independent main effect variables was minimized in a regression analysis, and the new score was scaled to have a minimum value of 0 and a maximum value of 1. In this method, multivariate generalized linear regression is applied to four new (0, 1) dependent variables that are created from the four largest scores on the original retinopathy severity scale: the i th new variable ($i = 1, 2, 3, 4$) was given the value 1 if the original severity score was $i + 1, 0$ otherwise. For these four new variables, we obtained the total sum of squares and cross-products matrix and the residual-error matrix for each of two models. To obtain the new score, the inverse of the total sum of squares and cross-product matrix for a model that included interactions plus residual error was postmultiplied by the sum of squares and cross-product matrix for a model that included only main-effect variables. We then calculated the vector corresponding to the largest characteristic root of this product matrix (dimension, 4×4), which yields the four largest rescaled scores; the original severity score of 1 is given a new score of 0. All regression analyses were performed with commercial software (SAS ver. 9.1 on a Windows platform; SAS, Cary, NC). The familial sibling correlation and its asymptotic SE were calculated for the rescaled scores (i.e., residuals), after partialing out significant covariate effects by using the SAGE ver. 5.0 program FCOR on a UNIX platform.²⁹ This program uses the

method of Keen and Elston³⁰ to estimate familial correlations and their standard errors without assuming multivariate normality. To calculate the standard errors, it relies on the fact that the sums of squares and cross products are asymptotically normally distributed. Doubling the sib-sib correlation (of the residuals) was used to estimate the b^2 of DR severity.

The association between DN and DR was assessed with a χ^2 test to examine whether the severity of retinopathy was dependent on nephropathy status. A measure of ordinal association, the Goodman and Kruskal γ coefficient, also considered whether the variable y (DR) tended to change as x (DN) changed. This measure classifies pairs of observations as concordant or discordant. A pair is concordant if the observation with the larger value of x also has the larger value of y . A pair is discordant if the observation with the larger value of x has the smaller value of y .

We then estimated the heritability of liability to PDR. The heritability of a dichotomous trait is often estimated on the assumption that there is an underlying normally distributed liability (and hence its genetic component is polygenic) and a threshold value of the liability determines the two classes of the dichotomy (i.e., PDR: yes or no). Using the threshold model of Falconer and Mackay,³¹ we estimated the b^2 as twice the sib correlation in susceptibility to PDR in our total sample, using the approximation in Mendel and Elston,²⁷ which corrects an error in Falconer's original paper. This model assumes that the prevalence of PDR is the same for both sibs in a pair.

Specifically, we estimated heritability of PDR, as described in the following equation. For the threshold method, we estimated the prevalence $P(1)$ of PDR and the probability $P(2)$ that both sibs of a pair have PDR. Let the i th sibship contain n_i diabetic sibs, $r_i \geq 0$ of whom have PDR. Therefore, to calculate $P(1)$, we listed all sibs and found the proportion that had PDR as follows:

$$P(1) = \frac{\# \text{ with PR}}{\# \text{ of sibs}} = \frac{\sum_i r_i}{\sum_i n_i} \quad (1)$$

To calculate $P(2)$, we listed all pairs of sibs, and found the proportion for which both members of the pair had PR:

$$P(2) = \frac{\sum_i r_i(r_i - 1)}{\sum_i n_i(n_i - 1)} \quad (2)$$

Then, the recurrence risk of PR to the sib of a proband with PDR is $P(2)/P(1)$.

We then calculated the sibling correlation in liability, ρ_{ss} , as in Mendel and Elston,²⁷ and let $b^2 = 2 \cdot \rho_{ss}$.

RESULTS

This report summarizes the data for 2368 diabetic subjects in 767 families enrolled in the FIND and FIND-Eye studies who had complete information on retinopathy and covariates. Participant characteristics are summarized in Table 1: 32.4% were DN probands ($n = 767$), 60.9% ($n = 1442$) diabetic siblings, and 6.7% ($n = 159$) other diabetic relatives. Diabetic siblings included 399 with DN, 391 with microalbuminuria, 413 without DN, and 238 indeterminate (<10 year diabetes duration without evidence of DN). There were 2183 diabetes-affected sib pairs and sibship sizes ranged from 2 to 10 per family (predominately 2 [$n = 442$] and 3 [$n = 211$] sibs per family). Proband had an average (SD) age of 58.5 (10.5) years at the time of photo/examination, whereas nonproband had an average (SD) age of 57.7 (12.1) years. Diabetes duration was 23.3 (8.6) years for probands and 14.3 (10.1) years for nonproband. Proband and nonproband had an average BMI of 30.2 (6.7) and 32.5 (7.8), respectively. The majority (50%) of the

TABLE 1. Participant Characteristics, by Proband Status

Characteristic	Proband (n = 767)	Nonproband (n = 1601)
Sex		
Female	404 (52.7)	1022 (63.8)
Ethnicity*		
African American	190 (24.8)	336 (21.0)
American Indian	70 (9.1)	198 (12.4)
European American	137 (17.9)	247 (15.4)
Mexican American	370 (48.2)	820 (51.2)
Clinical variables†		
Age at photo/exam (y)	58.5 (10.5)	57.7 (12.1)
Body mass index (kg/m ²)	30.2 (6.7)	32.5 (7.8)
Diabetes duration (y)	23.3 (8.6)	14.3 (10.1)

* N (%), frequency (percentage).

† Mean (SD).

enrolled subjects was Mexican American and 63.8% were female.

The prevalence of retinopathy in the overall sample was 73.4% (Table 2). The full spectrum of retinal changes was observed; 26.6% had no DR; 9.5%, 22.8%, and 7.5% had mild, moderate, or severe NPDR, respectively; and 33.4% had PDR. PDR was more frequent in the DN probands than in the nonprobands; whereas lack of DR was more common among nonprobands than among DN probands.

Table 3 reveals the distribution of DR severity based on DN phenotype. DN probands had significantly more severe DR than did those without DN. DN probands were more likely to have PDR (63.2%) than no DR (5.3%). Concordant siblings were slightly more likely to have PDR (39.6%) than no DR (18.3%); however, many had NPDR (41.9%). Discordant siblings more frequently had no DR (38.2%) compared with PDR (9.6%); whereas many had NPDR (52%). Most of the sibs with <10 years' diabetes duration had no DR (73.1%), compared with PDR (1.2%). However, some had NPDR (25.5%). Associations between DR severity and DN phenotypes were assessed with a χ^2 test and measurement of ordinal association. DR severity was significantly associated with nephropathy phenotype ($\chi^2 = 838.8985$, $df = 16$, $P < 0.0001$; $\gamma = 0.5536$, $P = 0.01$). DR severity was also associated with increasing serum creatinine (Scr) concentration ($\chi^2 = 658.1439$, $df = 20$, $P < 0.0001$ and $\gamma = 0.9168$, $P = 0.007$; Table 4). For example, among subjects whose Scr was <1.4 mg/dL, only 13.9% had PDR, and 39.6% had no DR. Among subjects whose Scr was 1.4 to 3 mg/dL, 44.3% had PDR, and only 14.4% had NPDR. Tables 3 and 4 demonstrate that the severity of DR increases in concert with deteriorating renal function.

After Fisher's method was applied,²⁸ the rescaled retinopathy severity scores were 0.000, 0.341, 0.688, 0.852, and 1.000.

A linear regression analysis was performed with these scores for the 2183 sibling pairs and the predictor variables (main effects plus one interaction term) included in the final model, together with their regression coefficients and standard errors (Table 5). Mexican-American ethnicity was associated with significantly higher mean rescaled retinopathy severity score, than African-American, American-Indian, or European-American ethnicity. Other factors significantly associated with higher DR severity scores were advanced age at the time of fundus photograph ($P < 0.0001$), HbA1c ($P < 0.0001$), increased duration of DM ($P < 0.0001$), and the presence of nephropathy ($P < 0.0001$). The men had more severe DR ($P < 0.0001$), an effect that was independent of ethnicity (Table 5). The association between the age at time of fundus photograph and higher severity scores was stronger in the women than in the men (i.e., sex \times age at photograph interaction; $P < 0.0001$).

After adjustment for the significant effects of the covariates in Table 5, the sib-sib correlation of the residuals was calculated on the basis of the total 2183 sibling pairs. The sib-sib correlation was 0.1358 (± 0.02) among all sib pairs and 0.1224 (± 0.01) when limited to the Mexican-American sib pairs. The broad-sense heritability was estimated to be approximately 27% and 24% in the total and Mexican-American samples, respectively. In addition, the prevalence $P(1)$ of PDR was 0.3425 and the probability $P(2)$ that both sibs in a pair had PDR was 0.0921. The sib recurrence risk for PDR in an individual whose sib was a proband having PDR was 0.2689 ($P(2)/P(1)$). The sibling correlation in liability (ρ_{ss}) was estimated as in Mendel and Elston²⁷ and b^2 was 0.2512 (i.e., $2 \cdot \rho_{ss}$). Therefore, the broad sense heritability of liability for PDR was 25% in the FIND-Eye sample.

DISCUSSION

Hyperglycemia and long duration of diabetes are widely recognized as major risk factors for the development of DR; however, inherited susceptibility may also play a role because retinopathy aggregates in families. Significant familial influences on the severity of DR in FIND-Eye families were detected in all ethnic groups. In another Mexican-American population, Hallman et al.¹¹ reported that the occurrence of severe DR in one sibling predicted increased risk for severe DR in other diabetic siblings, after adjustment for diabetes duration and glycemic control. In that report, severe DR exhibited strong familial aggregation, whereas the simple presence of DR did not.

As in this report, familial aggregation of DR severity in diabetic Mexican Americans from Starr County, Texas, was 70%, and nearly all enrolled subjects had some degree of DR.¹¹ The increased severity of DR among Mexican Americans in the FIND is consistent with other reports^{8,10,12} and the decreased

TABLE 2. Severity of Diabetic Retinopathy, by Proband Status

Retinopathy Severity Scale	Proband (n = 767)	Nonproband (n = 1601)	Total (n = 2368)
No diabetic retinopathy (ETDRS 10-12)*	41 (5.3)†	591 (36.9)	632 (26.6)
Mild NPDR (ETDRS 14-20)‡	29 (3.8)	199 (12.4)	228 (9.5)
Moderate NPDR ETDRS 35-43)	154 (20.1)	387 (24.2)	541 (22.8)
Severe NPDR (ETDRS 47-53)	57 (7.4)	115 (7.2)	172 (7.5)
Proliferative diabetic retinopathy (ETDRS \geq 60)	483 (63.0)	307 (19.2)	790 (33.4)
Ungradeable§	3 (0.4)	2 (0.1)	5 (0.2)

* Early Treatment of Diabetic Retinopathy Study Classification System.

† N (%), frequency (percentage).

‡ Nonproliferative diabetic retinopathy.

§ Assigned by FPRC when the quality of a photo is too poor or there is another eye condition that prevents accurate grading of photos.

TABLE 3. Distribution of Diabetic Retinopathy Severity and Associations between Nephropathy Status and Diabetic Retinopathy*

Nephropathy Status	No Diabetic Retinopathy	Mild NPDR†	Moderate NPDR	Severe NPDR	Proliferative Diabetic Retinopathy
Probands with DN (<i>n</i> = 764)	41 (5.37)‡	29 (3.80)	154 (20.16)	57 (7.46)	483 (63.22)
Sibs concordant for DN (<i>n</i> = 399)	73 (18.30)	33 (8.27)	98 (24.56)	37 (9.27)	158 (39.60)
Micro-albuminuric Sibs (<i>n</i> = 391)	126 (32.23)	40 (10.23)	119 (30.43)	31 (7.93)	75 (19.18)
Non-DN sibs (<i>n</i> = 413)	158 (38.26)	63 (15.25)	114 (27.60)	38 (9.20)	40 (9.69)
Indeterminate sibs (<10 year diabetes duration) (<i>n</i> = 238)	174 (73.11)	38 (15.97)	21 (8.82)	2 (0.84)	3 (1.26)

* $\chi^2 = 838.8985$, *df* = 16, *P* < 0.0001; and $\gamma = 0.5536$, *P* = 0.01. Data are expressed as the frequency (percentage).

† Nonproliferative diabetic retinopathy.

‡ Frequency (percentage).

severity of DR in African Americans in the FIND (relative to European Americans) has also been reported.⁹

It is clear that DR and DN frequently coexist, and DR is more severe in patients with severe DN. Patients with advanced DN in our report appeared to have far more severe DR, relative to those without DN, an effect that was particularly strong in Mexican-American families. The grade of DR also correlates strongly with microalbuminuria in the Pima population.³² Microalbuminuria appears to be a reliable marker for the presence of DR³³ and is particularly common among Mexican Americans. Jones et al.³⁴ reported that Mexican-American ethnicity was independently associated with the risk of microalbuminuria, after adjustment for diabetes, hypertension, cardiovascular, and kidney diseases. Other studies have noted that a significantly greater percentage of Mexican-Americans with type 2 DM have microalbuminuria than do non-Hispanic Caucasians.^{35,36} The mechanisms underlying the strong association between DR and DN, particularly in Mexican Americans, remain unclear. In the FIND study, we used a cross-sectional design, limiting our ability to determine causality. We are not aware of existing longitudinal reports in large cohorts that address this important question. Male gender was associated with severe DR in the FIND-Eye, consistent with the Starr County Mexican-American cohort. In Starr County, proportionately fewer men lacked DR and more had severe NPDR.³⁷ A similar protective effect of female gender has been observed on progression to micro- and macrovascular disease complications, especially for DN.³⁸ The mechanisms underlying this gender disparity could relate to differences in glomerular architecture or hemodynamics, variations in the production and activity of local cytokines and hormones, and/or the direct effect of sex hormones on kidney cells.^{39,40} Future studies should examine the role of sex in the development and progression of DR and DN in diabetic patients with different ethnic background.

In the WESDR, the widest and most prolonged population-based ophthalmologic survey, a higher prevalence of DR was

associated with longer durations of diabetes.⁴¹ In persons with type 1 diabetes of less than 5 year's duration, the prevalence of retinopathy was approximately 10%, and it ranged from 25% to 40% in individuals with type 2 diabetes. In addition, it has been demonstrated that for every 5-year increase in diabetes duration, the risk for DR increases 1.89 times.⁴²

We also demonstrated that increasing diabetes duration was strongly associated with more severe DR. Looker et al.³² reported that diabetes duration is related to high DR severity scores in the Pima Indians. Increased diabetes duration is also a risk factor for the presence of DR, but not necessarily for the presence of DN. The risk of development of DN after more than two decades of diabetes may slowly decline, especially in type 1 diabetes.⁴³ Glycemic control correlated significantly with the severity of DR (*P* = 0.0013) in the FIND-Eye study, but the correlation was low (correlation coefficient = 0.0690, SE = 0.0214), and it was not affected by the adjustment of HbA1c for diabetes duration. The significant correlation between HbA1c and DR severity is consistent with results from the Diabetes Complications and Control Trial (DCCT),¹³ the Los Angeles Latino Eye Study (LALES),⁴⁴ and the Multi-ethnic Study of Atherosclerosis (MESA).⁴⁵ The slight correlation maybe related to the large number of FIND-Eye participants with ESRD, because HbA1c does not accurately reflect glycemic control in ESRD.⁴⁶

A genome scan in Mexican-American families identified regions on chromosomes 3 and 12 that appear to harbor susceptibility loci for DR severity.³⁷ DR was also highly heritable in the Pima and evidence suggestive of linkage of DR to regions on chromosomes 3 and 9 was observed in this tribe.^{17,18,47} A recent genome-wide linkage analysis for DR detected evidence of linkage on the short arm of chromosome 1.³² Several candidate genes for DR susceptibility have been investigated, including the aldose reductase receptor (*ALR2*), nitric oxide synthase (*NOS*), receptor for advanced glycation end products (*RAGE*), vascular endothelial growth factor (*VEGF*), intercellular adhesion molecule 1, β 3-adrenergic receptor, angiotensin converting enzyme (*ACE*), and the α 2 β 1-integrin genes.^{48,49}

TABLE 4. Distribution of Retinopathy Severity by Serum Creatinine Concentration and Associations between Serum Creatinine Concentration and Diabetic Retinopathy*

Serum Creatinine Concentration (mg/dL)	No Diabetic Retinopathy	Mild NPDR†	Moderate NPDR	Severe NPDR	Proliferative Diabetic Retinopathy
<1.4 (<i>n</i> = 1256)	498 (39.65)‡	157 (12.50)	326 (25.96)	100 (7.96)	175 (13.93)
1.4–3 (<i>n</i> = 221)	32 (14.48)	14 (6.33)	57 (25.79)	20 (9.05)	98 (44.34)
>3–5 (<i>n</i> = 46)	5 (10.87)	1 (2.17)	9 (19.57)	3 (6.52)	28 (60.87)
>5–7 (<i>n</i> = 13)	0 (00.0)	1 (7.69)	4 (30.77)	0 (0.00)	8 (61.54)
>7 (<i>n</i> = 23)	0 (0.00)	0 (0.00)	4 (17.39)	3 (13.04)	16 (69.57)
ESRD (<i>n</i> = 646)	37 (5.73)	30 (4.64)	106 (16.41)	39 (6.04)	434 (67.18)

* $\chi^2 = 658.1439$, *df* = 20, *P* < 0.0001 and $\gamma = 0.9168$, *P* = 0.007.

† Nonproliferative diabetic retinopathy.

‡ Frequency (percentage).

TABLE 5. Linear Regression of the Rescaled Retinopathy Severity Scores

Variable	Regression Coefficient	Standard Error	P
Intercept	-2.305	0.535	<0.0001
Main effects			
African American*	-0.507	0.065	<0.0001
American Indian*	-1.235	0.092	<0.0001
European American*	-0.374	0.073	<0.0001
Nephropathy status†	1.102	0.061	<0.0001
Sex male	1.4045	0.280	<0.0001
HbA1c	0.069	0.0214	<0.0001
Age at photo	0.126	0.018	<0.0001
Diabetes duration (Age) ²	0.134	0.008	<0.0001
(Diabetes duration) ²	-0.001	0.0004	<0.0001
(Diabetes duration) ²	-0.002	0.0005	<0.0001
Interaction terms‡			
Sex × age at photo	-0.018	0.005	<0.0001

* Baseline ethnicity, Mexican-American.

† Nephropathy status: 1, has nephropathy; 0, does not have nephropathy.

‡ All other interactions terms were non-significant, $P > 0.1$.

In the FIND-Eye study, DR and DN correlated highly and DR was partially under familial influence. However, one potential limitation is that “heritability” could be overstated because families were selected to have siblings concordant for nephropathy. Future studies are needed to replicate our findings. Several susceptibility loci for DN have been identified in the FIND,⁵⁰ and genome-wide linkage analyses for DR phenotypes will be performed in the near future. The results of these analyses have the potential to localize and identify susceptibility genes that have trait-specific influences DN or DR, as well as common genetic influences involving both DR and DN. It is possible that susceptibility genes with direct relevance to severity of DR may be detected. Identification of genetic determinants of DR will improve our understanding of the development and progression of this severe disorder, one of the leading causes of visual impairment in the United States. Progress in identification may lead to improved prevention and treatment strategies in individuals who are at high risk for development of diabetic eye disease.

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APPENDIX

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