

A genome-wide linkage scan for diabetic retinopathy susceptibility genes in Mexican Americans with type 2 diabetes from Starr County, Texas

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Additional information can be found in an online appendix.

Abstract

We conducted a genome-wide linkage scan for genes contributing to retinopathy risk, using 794 diabetes cases from 393 Mexican American families from Starr County, Texas, having at least two diabetic siblings. The sample included 567 retinopathy cases comprising 282 affected sibling pairs. Retinopathy was classified as: none, early non-proliferative, moderate-to-severe nonproliferative, or proliferative. Using 360 polymorphic markers (average spacing: 9.4 cM), we conducted nonparametric linkage analysis, followed by ordered-subset analysis (OSA) ranking families by average age of diabetes diagnosis. For any retinopathy, the highest LOD scores including all families were on chromosomes 3 (2.41 at 117 cM) and 12 (2.47 at 15.5 cM). OSA LOD scores > 2 for any retinopathy occurred on chromosomes 12 (4.47 at 13.2 cM), 15 (3.65 at 100.6 cM), and 20 (2.67 at 54.1 cM). OSA LOD scores > 2 for either moderate-to-severe nonproliferative or proliferative retinopathy occurred on chromosomes 5 (2.53 at 11.2 cM), 6 (2.28 at 30.6 cM), and 19 (2.21 at 100.6 cM). Thus, unconditional linkage analysis revealed suggestive evidence of linkage with retinopathy on two chromosomes, while ordered-subset analysis revealed strong evidence of linkage on two chromosomes, and suggestive evidence on four. Candidate genes were identified in most implicated regions.

Diabetic retinopathy, a frequent complication of both type 1 and type 2 diabetes, is the fifth most common cause of legal blindness in the United States (1). Some degree of retinopathy occurs in virtually all type 1 and 60% of type 2 diabetes patients affected 20 years or more, though severe proliferative retinopathy is more frequent in type 1 diabetes. The underlying causes of diabetic retinopathy have not yet been elucidated, though tight control of hyperglycemia can retard its development and progression (1-4).

While studies of familial aggregation of diabetic retinopathy suggest that genes may influence either its onset (5, 6) or its severity (7, 8), most studies of the genetics of diabetic retinopathy have involved candidate genes. Polymorphisms in several genes have been associated with diabetic retinopathy, though few associations have been replicated in multiple populations (9). Exceptions include aldose reductase (10-17) and the insertion/deletion polymorphism of the angiotensin I-converting enzyme (18, 19), though the latter association has been questioned (20).

In Pima Indians, a genomic scan revealed evidence of linkage between regions on chromosomes 3 and 9 and the occurrence of retinopathy in 136 affected siblings (103 affected pairs) with type 2 diabetes, with a maximum multipoint LOD score of 1.46 for the region on chromosome 9 (21). We here report the results of a genome-wide linkage scan for the occurrence and severity of retinopathy in Mexican American families from Starr County, Texas, having at least two siblings affected with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects: Mexican American families from Starr County, Texas, having two or more siblings with type 2 diabetes were eligible for the study. Diabetes classification was based on earlier National Diabetes Data Group guidelines (1979) wherein individuals currently treated for diabetes, having fasting glucose ≥ 140 mg/dl on more than one occasion, or having an abnormal glucose tolerance test were considered to have diabetes. A diagnosis of type 2 diabetes was excluded if age at diagnosis was less than 30 years, BMI was less than 30, and insulin had been used continuously since diagnosis. Subjects were enrolled through the Family Blood Pressure Program, as previously described (22).

Markers: The total number of markers typed was 360, covering the 22 autosomes at an average spacing of 9.38 cM (SD: 4.13). The minimum distance between any two markers was 0.55 cM on chromosome 18; the maximum distance was 32.97 cM, on chromosome 14.

Retinopathy Grading: Stereoscopic color fundus photographs of 7 standard fields of each eye were scored using the Early Treatment Diabetic Retinopathy Study adaptation of the modified Airlie House classification system (23), as described previously (8). Retinopathy was classified as: none, early nonproliferative (NPDR-E), moderate-to-severe nonproliferative (NPDR-S), or proliferative (PDR).

Analyses: Linkage analyses were performed with GeneHunter Plus, using the linear model and S_{pairs} , the number of pairs of alleles shared identical-by-descent by affected pedigree

members (24). In addition, we used ordered-subset analysis (OSA), as implemented in the OSA program (25), to look for homogeneous subsets of families with maximal evidence of linkage to a given chromosomal region. The mean age of type 2 diabetes diagnosis within families was used to rank families for ordered subset analysis. Analyses were also conducted with families ordered by mean diabetes duration, calculated as the difference between the age at diagnosis with type 2 diabetes and the age when examined for retinopathy. However, since actual ages of onset of neither diabetes nor retinopathy could be determined precisely, using diabetes duration estimated from ages of diabetes diagnosis and examination for retinopathy would compound the degree of uncertainty in the analyses. Therefore, we report results for the ordered-subset analyses using age of diabetes diagnosis. The difference between the maximum LOD score in the subset of families identified by OSA and the LOD score at the same position in the full set of families was evaluated by permutation testing; a significant p-value denotes a maximum OSA LOD score significantly greater than the corresponding unconditional LOD score (25). In separate analyses, individuals were considered affected if they had any retinopathy (NPDR-E, NPDR-S, or PDR) or more severe retinopathy (NPDR-S or PDR).

In comparing covariates among retinopathy classes, logistic regression with generalized estimating equations was used to account for the correlations among siblings. These analyses were performed using SAS, Version 8 (SAS Institute, Cary, NC).

RESULTS

Subjects were drawn from 415

sibships identified during a study of the genetics of type 2 diabetes. No subjects were available for retinopathy examinations from 22 families (5.1%). The remaining 393 families contained from one to eleven offspring, with 391 (99.5%) having at least two. A total of 794 individuals were examined for retinopathy, of whom 567 (71.4%) were affected; genotype data were available for another 791 sibship members who were not examined for retinopathy. In 100 families (25.4%) only one individual was available to be examined for retinopathy. Of the 293 families in which at least two members were examined, 16 (5.5%) had no members affected with retinopathy, 100 (34.1%) had one member affected, 143 (48.8%) had two members affected, 27 (9.2%) had three members affected, three (1.0%) had four members affected, and four (1.4%) had five members affected, yielding 282 affected pairs from 177 families. For more severe retinopathy (NPDR-S or PDR), 74 affected pairs from 52 families were available, while for PDR, only 8 affected pairs from 8 families were available (Supplementary Table 1). In our sample, families containing only pairs discordant for retinopathy contributed no linkage information.

Table 1 shows characteristics of the subjects according to retinopathy grade. Proportionately fewer males than expected had no retinopathy, while more males than expected had severe non-proliferative retinopathy. Observed numbers of males and females with proliferative retinopathy (36 and 52, respectively) closely matched expected numbers (34 and 54, respectively). Systolic blood pressure and plasma cholesterol levels tended to increase with retinopathy severity, while body mass index tended to decrease, and was highest among those with no retinopathy. Fasting blood glucose, HbA1c,

and plasma triglyceride levels were highest in subjects with moderate-to-severe nonproliferative retinopathy. As expected, there was a clear positive relationship between duration of diabetes and the presence and severity of retinopathy. Subjects with more severe retinopathy, either proliferative or nonproliferative, were diagnosed with diabetes at markedly younger ages (46 years), on average, than those with less severe retinopathy (50 years) or no retinopathy (52 years). As expected, age at diabetes diagnosis and duration of the disease at the time of retinopathy examination were negatively correlated ($\rho = -0.40$; $p < 0.0001$).

Figure 1 shows results for the unconditional linkage analyses for retinopathy of any severity, while Table 2 shows all unconditional LOD scores greater than 1.00 for any retinopathy and for more severe retinopathy (NPDR-S or PDR). For retinopathy of any severity, the highest LOD scores occurred on chromosomes 3 (LOD score of 2.41 at 117.0 cM) and 12 (LOD score of 2.47 at 15.5 cM); no other unconditional LOD scores exceeded 1.24 (chromosome 1, 45.3 cM). While the LOD scores on chromosomes 3 and 12 do not reach the level of chromosome-wide significance, they do provide suggestive evidence of linkage (26).

Table 3 shows results of the ordered-subset analyses with families ranked according to average age of type 2 diabetes diagnosis. For retinopathy of any degree, subsets of families yielded significantly increased LOD scores on chromosomes 12, 15, 18, and 20. For chromosomes 12, 15, and 20, maximum LOD scores occurred with families ranked from highest to lowest average age of diabetes diagnosis. On chromosome 12, the maximum OSA LOD score was 4.47 at 13.2 cM, compared to an

unconditional LOD score of 2.47 ($p = 0.018$). On chromosome 15, the maximum OSA LOD score was 3.65 at 100.6 cM, compared to an unconditional LOD score of 0.99 ($p = 0.030$). On chromosome 20, the maximum OSA LOD score was 2.67 at 54.1 cM, compared to an unconditional LOD score of 0.00 ($p = 0.004$). On chromosome 18, the peak OSA LOD score was obtained with families ranked from lowest to highest average age of diabetes diagnosis (OSA LOD = 1.90 at 99.0 cM; unconditional LOD = 0.06; $p = 0.033$).

The maximum unconditional LOD score for more severe retinopathy was 1.40, on chromosome 3 at 117.0 cM, the same location at which a LOD score of 2.41 was obtained for retinopathy of any degree. Another peak unconditional LOD score above 1.0 (1.29) for more severe retinopathy occurred on chromosome 3 at 9.4 cM. LOD scores above 1.0 for more severe retinopathy also occurred at the distal end of chromosome 2 (1.11 at 260.6 cM) and on chromosome 12 (1.03 at 100.5 cM). Ordered-subset analyses of more severe retinopathy, again ranking families by average age of type 2 diabetes diagnosis, yielded significantly increased LOD scores on chromosomes 5 (OSA LOD of 2.53 at 11.2 cM vs. unconditional LOD of 0.15; $p = 0.013$), 6 (OSA LOD of 2.28 at 30.6 cM vs. unconditional LOD of 0.62; $p = 0.041$), and 19 (OSA LOD of 2.21 at 100.6 cM vs. unconditional LOD of 0.28; $p = 0.037$).

CONCLUSIONS

Though evidence has been accumulating that genetic factors can influence either the occurrence or severity of diabetic retinopathy, it may be difficult to separate the genetics of diabetes from the

genetics of its complications. But it is important that we make the effort, because while treating the underlying disease may ameliorate its complications, treating the complications may be just as important in reducing the personal, social, and economic burdens of the disease. Understanding the genetic factors that either contribute to the development of retinopathy or increase its severity may allow us to move toward treatment of the underlying biology of the condition, rather than relying on palliative treatments, such as laser photocoagulation, that are aimed at its symptoms.

Our study represents a step toward this goal. Our results are consistent with evidence from earlier studies of familial aggregation (5-8), and with prior linkage (21) and association (9) studies, suggesting that genes that influence the risk of diabetic retinopathy exist. Furthermore, our findings provide evidence that such genes may be distinguishable from those that influence the risk of diabetes itself: None of the LOD scores greater than 2.0 reported here for retinopathy coincided with any LOD score peaks for type 2 diabetes in this same population (data not shown). This may have implications for all diabetic retinopathy, if the same genes that influence risk of retinopathy in type 2 diabetes also affect retinopathy associated with other forms of diabetes, such as type 1 diabetes and maturity-onset diabetes of the young.

Even though our study represents the largest linkage analysis of diabetic retinopathy yet reported, none of the unconditional LOD scores reached the level of genome-wide significance (26). Nonetheless, our findings are strongly suggestive of linkage between retinopathy and several chromosomal regions, particularly in

conjunction with the presence of several strong candidate genes in chromosomal regions implicated by our analyses (Table 4; citations refer to Supplementary Table 2 online). Considering both unconditional and ordered-subset analyses, the strongest evidence of linkage with retinopathy involved the proximal end of chromosome 12. The unconditional LOD score for any retinopathy of 2.47 at 15.5 cM was the highest unconditional score on any chromosome, while the peak LOD score of 4.47 at 13.2 cM was the highest score for any of the ordered-subset analyses; the region under these peaks encompasses approximately 24 cM. As shown in Table 4, several candidate genes potentially involved with either type 2 diabetes, diabetic retinopathy, or both occur in this region, including WNT5B, TULP3, and GNB3. At least two genes associated with hypertension, WNK1 and SCNN1A, also occur in this region. The OLR1 gene has been associated with hypertensive vascular damage and may be involved in choroidal neovascularization in age-related macular degeneration, suggesting that it could play a similar role in proliferative diabetic retinopathy.

The second-highest unconditional LOD score for any retinopathy (2.41) occurred on chromosome 3 at 117 cM, with a one-LOD support region covering nearly 21 cM. No significantly higher score in this region was found with ordered-subset analysis. Within the core of this region, at least two genes known to be involved in retinal diseases occur, PROS1 and ARL6, as well as two others, ROBO2 and IMPG2, that are involved in retinal development or function. Another gene involved in retinal development, MITF, is proximal to the core region, while GUCA1C, involved in retinal photoreceptor activity, is distal to it. It should

be noted that this region differs from the region on chromosome 3 that produced a peak LOD score of 1.36 for retinopathy in Pima Indians (21).

Because age at diabetes diagnosis is likely to be inversely correlated with the duration of the disease in those examined for retinopathy, maximum LOD scores obtained when ranking families from higher to lower age at diagnosis may implicate variants that contribute to more rapid development of retinopathy. Conversely, maximum LOD scores obtained when ranking families from lower to higher mean ages of diabetes diagnosis may implicate variants that are associated with a milder course of development of retinal damage.

On chromosome 15, ordered-subset analysis yielded a maximum LOD score of 3.65 at 100.6 cM ($p=0.031$); the highest unconditional LOD score in this region was 1.16 at 108.3 cM. Among potential candidates involved in retinal biology that occur in or near this region are IGF1R and RGMA. The IDDM3 gene is near this region, though the gene itself has not yet been identified (27).

On chromosome 20, ordered-subset analysis for any retinopathy produced a peak LOD score of 2.67 at 54.1 cM, significantly different ($p = 0.004$) from the unconditional LOD score of 0 at this position. Several genes in the region are known to be involved in retinal biology or retinal disease, including the transglutaminases, TGM2 and TGM3, and KCNS1. Several other genes in the region may be associated with various aspects of insulin resistance or type 2 diabetes, including E2F1, ASIP, and HNF4A, the gene for type 1 maturity-onset diabetes of the young. The putative NIDDM3 locus is distal to the

implicated region (28-31).

The ordered-subset analyses for more severe retinopathy produced LOD scores greater than 2.00 on chromosomes 5 (2.53 at 11.2 cM), 6 (2.22 at 104.7 cM), and 19 (2.21 at 100.6 cM). Within the implicated region of chromosome 5, we identified no obvious candidate genes for retinopathy, though several (GDNF, MCDR3, SLC1A3) occur somewhat distal to this region (32-34). On chromosome 6, candidates in the implicated region include EDN1 and GMNN. On chromosome 19, the peak occurred at the distal end of the chromosome. Within 20 cM of the end, however, is PRPF31, the gene for autosomal dominant retinitis pigmentosa type 11; also in this region is FIZ1, which interacts with NRL, a gene involved in another form of retinitis pigmentosa.

That the strongest evidence for genetic linkage was found in analyses of retinopathy of any severity is interesting, since previous analyses in this population indicated that more severe retinopathy, but not retinopathy *per se*, showed familial aggregation (8). These seemingly contradictory findings may be due to the low statistical power of nonparametric linkage analyses relative to that of the association analyses used to assess familial aggregation (35); the set of subjects having any degree of retinopathy is much larger than the subsets with either more severe nonproliferative or proliferative retinopathy, or proliferative retinopathy alone. Also, there is a distinction between testing whether a phenotype shows familial aggregation overall, and testing whether certain markers are shared more frequently by individuals with a given phenotype. Heritability estimates for quantitative traits have been found to show little correlation with measures of association in genomic scans (36), suggesting that

measures of familial aggregation of disease could well be poorly correlated with measures of linkage. In addition, the very high prevalence of retinopathy in our family sample (~70%) may help obscure evidence of familial aggregation of overall retinopathy, but have less effect on tests of marker sharing, especially among more homogeneous subsets of families.

In summary, both unconditional and ordered-subset linkage analysis identified several regions possibly harboring retinopathy susceptibility loci. Strong candidate genes, many of them specific to the retina or associated with other retinal pathology, were identified in most of these regions, strengthening the case that genes can affect susceptibility to diabetic retinopathy. Such genes, however, may have pathological effects in the retina only when the underlying pathology of diabetes is present. Inasmuch as most morbidity and mortality from diabetes

are attributable to its complications, analyses that focus on the complications of diabetes, as here, may be an important adjunct to studies of the genetics of diabetes.

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Table 1. Selected characteristics of subjects, by retinopathy grade.				
	Retinopathy Grade			
	None	NPDR-E	NPDR-S	PDR
Sex (M/F)*	74/153	110/192	90/87	36/52
Age at DM Diagnosis (yrs)‡	52.0 ± 10.3	49.9 ± 11.0	46.2 ± 10.4	46.1 ± 10.6
DM Duration (yrs)‡	5.8 ± 5.2	9.4 ± 7.6	12.0 ± 6.7	15.2 ± 9.0
BMI (kg/m ²)†	32.8 ± 6.2	31.6 ± 6.2	30.8 ± 5.7	30.6 ± 6.1
Fasting Glucose (mg/dL)*	172.7 ± 57.3	191.6 ± 65.5	203.1 ± 79.4	182.8 ± 85.3
HbA1c (%)‡	10.1 ± 2.9	11.0 ± 3.1	12.3 ± 3.0	10.9 ± 3.2
Diastolic BP (mmHg)	73.7 ± 10.5	72.2 ± 9.9	73.5 ± 11.4	72.6 ± 11.3
Systolic BP (mmHg)‡	127.4 ± 19.0	130.8 ± 18.2	132.9 ± 22.3	139.5 ± 23.9
Cholesterol (mg/dL)‡	211.0 ± 43.2	210.9 ± 44.4	223.4 ± 50.2	236.1 ± 58.2
Triglycerides (mg/dL)*	194.6 ± 102.3	202.5 ± 109.8	230.2 ± 125.8	213.9 ± 111.6
Values given as means ± SD. * Significant at p < 0.01. † Significant at p < 0.001. ‡ Significant at p < 0.0001.				

Table 2. Unconditional LOD scores of 1.0 or greater.						
Chr	LOD	Position (cM)	Nearest Marker	One-LOD Interval	p	Retinopathy
1	1.240	45.3	GGAT2A07	18.2 - 71.7	0.0169	Any
2	1.105	260.6*	AFM112yd4	219.4 - 260.6*	0.0241	NPDR-S/PDR
3	1.292	9.4	GATA22G12	0 - 26.4	0.0147	NPDR-S/PDR
3	1.402	117.0	GATA68D03	96.4 - 130.6	0.0111	NPDR-S/PDR
3	2.413	117.0	GATA68D03	101.9 - 122.7	0.0009	Any
7	1.020	33.1	GATA41G07	8.7 - 103.8	0.0302	Any
12	2.469	15.5	GATA49D12	0 - 24.0	0.0007	Any
12	1.029	100.5	GATA85A04	75.2 - 123.8	0.0295	NPDR-S/PDR
15	1.070	78.4	ATA28G05	28.7 - 122.1*	0.0264	Any
15	1.160	108.3	GATA22F01	32.7 - 122.1*	0.0208	Any
* Distal end of chromosome						

Table 3. Results of ordered-subset analysis with families ranked by mean age of diabetes diagnosis.

Chr	Peak OSA LOD	Position (cM)	Nearest Marker	One-LOD Interval	Direction	Unconditional LOD	Families (used/total)	p	Retinopathy
5	2.528	11.2	GATA84E11	0.0 - 13.4	L -> H	0.145	24/52	0.0129	NPDR-S/PDR
6	2.283	30.6	GATA29A01	12.2 - 42.2	L -> H	0.616	21/52	0.0410	NPDR-S/PDR
12	4.470	13.2	GATA49D12	0.0 - 22.9	H -> L	2.468	73/177	0.0180	Any
15	3.652	100.6	GATA73F01	95.0 - 103.6	H -> L	0.987	38/177	0.0300	Any
18	1.902	99.0	GATA7E12	90.0 - 111.7	L -> H	0.059	17/177	0.0330	Any
19	2.210	100.6	Mfd238	84.5 - 100.6*	L -> H	0.276	27/52	0.0365	NPDR-S/PDR
20	2.671	54.1	GATA42A03	50.6 - 58.2	H -> L	0.000	29/177	0.0042	Any

* Distal end of chromosome

Table 4. Potential candidate genes in regions under linkage peaks with unconditional or ordered-subset LOD scores ≥ 2.0 .

Chr	Gene	Comment	Reference
3	ROBO2	Retinal development	S1
	PROS1	Venous thrombosis; retinopathy of prematurity	S2-S3
	ARL6	Bardet-Biedl Syndrome w/ retinal dystrophy	S4-S5
	IMPG2	Retinal interphotoreceptor matrix proteoglycan	S6
6	EDN1	Affects retinal blood flow	S7-S8
	GMNN	Development of retinal precursor cells	S9
12	WNT5B	Adipogenesis; type 2 diabetes	S10
	TULP3	TUB/TULP1/TULP2 family (retinal degeneration)	S11-S13
	GNB3	Hypertension and obesity	S14-S15
	WNK1	Hypertension (pseudohypoaldosteronism)	S16
	SCNN1A	Hypertension (pseudohypoaldosteronism)	S17-S18
	ING4	Angiogenesis	S19
	OLR1	Hypertensive vascular damage; choroidal neovascularization in age-related macular degeneration	S20-S21
15	IGF1R	Post-hypoxia retinal neovascularization	S22
	RGMA	Axonal placement in retinal development	S23
	NR2F2	Angiogenesis (primarily venous)	S24
	MEF2A	CVD; functions in arterial endothelium	S25
	IDDM3	(Gene not yet identified)	S26
19	PRPF31	Autosomal dominant retinitis pigmentosa 11	S27
	FIZ1	Interacts with NRL retinitis pigmentosa gene	S28
20	TGM2/3	Proliferative vitreoretinopathy	S29
	KCNS1	Expressed only in neurons, incl. retinal neurons	S30

	E2F1	Adipogenesis	S31
	ASIP	Adipocyte lipid metabolism and obesity	S32
	HNF4A	Type 1 maturity-onset diabetes of the young	S33

Figure 1. LOD score curves for unconditional linkage analyses of retinopathy of any severity.

