Award Number: W81XWH-07-1-0313

TITLE: Children's Hospital of Pittsburgh and Diabetes Institute of the Walter Reed Health Care System Genetic Screening in Diabetes: Candidate Gene Analysis for Diabetic Retinopathy

PRINCIPAL INVESTIGATOR: Robert A. Vigersky, COL MC

CONTRACTING ORGANIZATION:
TRUE Research Foundation
San Antonio, TX 78217

REPORT DATE: a l·è нлмл

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command

Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
May нлмл	Final	но ! tw нллт π нн ! tw нлмл

4. Title

Children's Hospital of Pittsburgh and Diabetes Institute of the Walter Reed Health Care System Genetic Screening in Diabetes: Candidate Gene Analysis for Diabetic Retinopathy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
² ум · ² l πлттмπлломо

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Robert A. Vigersky, COL MC
vigersky@na.amedd.army.mil

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
T.R.U.E Research Foundation
San Antonio, TX 78217

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The hypothesis to be tested was that there are allelic variations of some genes that make the development of diabetes-related complications more likely in patients who carry them than those who do not. The 3 major complications to be evaluated were diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy. This was an observational study in which the investigators obtained DNA samples from the blood of patients with one or more of these complications and from as many their first-degree relatives as possible for testing in the laboratory of Dr. Massimo Trucco in the Rangos Research Center at the Children's Hospital of Pittsburgh (CHOP). Dr. Trucco is an internationally known immunologist and respected leader in genetic research in diabetes. He evaluated these samples by studying candidate genes selected *a priori* and testing for transmission/disequilibrium – a standard for analysis of linkage between a candidate gene and a specific disease.

15. SUBJECT TERMS

None provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER**	**19a. NAME OF RESPONSIBLE PERSO**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Introduction

Although deaths today from the acute effects of diabetes are rare, the associated vascular, retinal, neurological and renal complications are responsible for high levels of morbidity and mortality in diabetes. However, it has been observed that only a subset of people with diabetes appear to be susceptible to the development of diabetes-related complications, i.e., nephropathy, autonomic neuropathy, and retinopathy, and there is data to suggest that there is a genetic component to this increased susceptibility. This investigation tested the hypothesis that there are allelic variations of some genes that make the development of diabetes-related complications more likely in patients who carry them than in those who do not. Initial emphasis was on the examination of candidate gene analysis in families for diabetic nephropathy, autonomic neuropathy, and retinopathy.

Body

This study, "Genetic Screening in Diabetes", was an observational study in which COL Vigersky and his research team obtained DNA samples from the blood of patients with type 1 or 2 diabetes who had at least one of three diabetic complications (as specified in SF298) and from as many of their first-degree relatives as possible for genetic testing. The study was conducted at WRAMC for DEERS-eligible subjects and at the White Flint Professional Building in Kensington, Maryland for non-DEERS-eligible subjects. All subjects completed a medical history, a quality of life questionnaire, a physical examination, blood and urine sampling and analysis, and additional procedures to rule out diabetes and the presence or absence of the three diabetes-related complications that are being studied. All blood samples will be typed and examined to evaluate if there are reasonable candidate genes that contribute to the genetic susceptibility and/or development of diabetic nephropathy, neuropathy, and retinopathy. Sixty-one probands and 62 family members completed the study.

Key Research Accomplishments

- Samples from the 124 consented subjects have been sent to the Rangos Research Center, University of Pittsburgh, Pittsburgh, PA for genetic analysis.
- During the period of this report, the RRC focused their effort on recruitment of additional subjects for the Type 1 Diabetic Nephropathy (T1DN) study. As a result, they have identified a genetic signal on Chromosome 13q with a p-value for T1DN less than 2E-07. While this is an excellent p-value for association it does not exceed the Bonferroni correction for multiple testing. In the genome-wide association scan (GWAS) that was used to compare the genetics of T1DN cases and T1D controls they originally genotyped roughly 500,000 single nucleotide polymorphisms. This number of independent tests for gene association resulted in a threshold for

genome-wide significance of 1E-07 (i.e. 0.05 divided by 500,000). Their value is close but is not yet significant.

- In order for the observed p-value to become significant RRC needs to recruit additional subjects to the study. The ideal cohort would be an independent group of T1DN cases and T1D controls that is roughly the same number (N=1,000 cases and N=1,000 controls) as was used in the original genome-wide association study (GWAS).
- RRC needs approximately 800 DN samples to confirm the results, but combined efforts from all sites have resulted in less than 200 samples.
- RRC will use samples sent from WRAMC to confirm their findings for T!DN and may use the samples later to identify possible associations between specific genes and diabetic retinopathy and neuropathy.

Reportable Outcomes & Conclusions

- There are no findings or conclusions to date from the samples we have sent to Rangos Research Center,

Summary

- Enrollment for this study was closed on 3 August 2009.
- Since June 2008, the study had been conducted under two no cost extensions. The first was submitted in June 2008 and approved in October 2008, the second was submitted in February 2009 and approved in March 2009.
- Attempts to obtain additional funding for this study were unsuccessful. In July, 2009, it was determined that the RRC needed far more samples than WRAMC was likely to provide. Given the lack of funding and the prior enrollment rate, the PI made the decision to close the study. The last proband was enrolled and completed the study on August 3, 2009.

References

American Diabetes Association: Standards of medical care for patients with diabetes mellitus. Diabetes Care. 21 (Suppl 1):s23-S31, 1998

Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type I (insulin dependent) diabetes: an epidemiologic study. Diabetologia. 25:496-501, 1983.

Ballard DJ, Humphrey LL, Melton J, Frohnert PP, Chu C-P, O'Fallon WM, Palumbo PJ: Epidemiology of persistent proteinuria in type II diabetes mellitus. Population based study in Rochester, Minnesota. Diabetes. 37:405-412, 1988.

Barzilay J, Warram JH, Bak M, Laffel LMB, Canessa M, Krolewski AS: Predisposition to hypertension: risk factor for nephropathy and hypertension in IDDM. Kidney International. 41:723-730, 1992.

Benjafield A, Glenn C, Wang X, Colagiuri S, and Morris B: TNFRSF1B in genetic predisposition to clinical neuropathy and effect on HDL cholesterol and glycosylated hemoglobin in Type 2 diabetes. Diabetes Care 24: 753-757, 2001.

Borch-Johnsen K, Norgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving H-H: Is diabetic nephropathy an inherited complication? Kidney International. 41:719-722, 1992.

Brancati FL, Whittle JC, Whelton PK, Seidler AJ, Klag MJ: The excess incidence of diabetic end-stage renal disease among Blacks. J Am Med Assoc. 268:3079-3084, 1992.

Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, Westlake L. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. BMJ 305:160-4, 1992.

Brownlee M: Glycation and diabetic complications. Diabetes. 43:836-841, 1994

Cheung VG, Gregg JP, Gogolin-Ewens KJ, Bandong J, Stanley CA, Baker L, Higgins MJ, Nowak NJ, Shows TB, Ewens WJ, Nelson SF, Spielman RS: Linkage disequilibrium mapping without genotyping. Nature Genetics. 18:225-230, 1998.

Chowdhury TA, Kumar S, Barnett AH, Bain SC: Nephropathy in type 1 diabetes: the role of genetic factors. Diabetic Medicine. 12:1059-1067, 1995.

Concannon P, Gogolin-Ewens KJ, Hinds DA, Wapelhorst B, Morrison VA, Stirling B, Mitra M, Farmer J, Williams SR, Cox NJ, Bell GI, Risch N, Spielman RS: A second-generation screen of the human genome for susceptibility to type 1 (insulin-dependent) diabetes mellitus (IDDM). Nature Genet 19:292-296, 1998.

Cowrie CC, Port, FK, Wolfe RA, Savage PJ, Moll PP, Hawthorne VM: Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. N Engl J Med. 321:1074-1079, 1989.

Crino PB, Trojanowski JQ, Dichter MA, Eberwine J: Embryonic neuronal markers in tuberous sclerosis: single-cell molecular pathology. Proc Natl Acad Sci. USA. 93:14152-14157, 1996.

Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 329: 977-986, 1993.

DeRisi J, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM: Use of a cDNA microarray to analyze gene expression patterns in human cancer. Nature Genetics. 14:457-460, 1996.

DeRisi J, Iyer VR, Brown PO: Exploring the metabolic and genetic control of gene expression on a genomic scale. Science. 278:680-686, 1997.

Doria A, Warram JH, Krolewski AS: Genetic susceptibility to nephropathy in insulin-dependent diabetes: from epidemiology to molecular genetics. Diabetes/Metabolism Reviews. 11:287-314, 1995.

Earle K, Walker J, Hill C, Viberti G: Familial clustering of cardiovascular disease patients with insulin-dependent diabetes and nephropathy. N Engl J Med.. 326:673-677, 1992.

Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs – an extension of the modified Airlie House Classification: ETDRS Report Number 10. Ophthalmology 98:786-806, 1991.

Ermolaeva O, Rastogi M, Pruitt K et al: Data management and analysis in gene expression arrays. Second workshop on methods and applications of DNA microarray technology, Tuscon, Arizona, 1998.

Ewens KG, George RA et al: Assessment of 115 Candidate Genes for Diabetic Nephropathy by Transmission/Disequilibrium Test . Diabetes 54: 3305-3318, 2005.

Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care 17:1281-1289, 1994.

Fioretto P, Steffes MW, Sutherland DE, Maurer M: Sequential renal biopsies in insulin-dependent diabetic patients: structural factors associated with clinical progression. Kidney International. 48:1929-1935, 1995.

Fodor SPA, Read LJ, Pirrung MC et al.: Light-directed, spatially addressable parallel chemical synthesis. Science. 251:761-773, 1991.

Freedman BI, Tuttle AB, Spray BJ: Familial predisposition to nephropathy in African-Americans with non-insulin-dependent diabetes mellitus. Am J Kid Dis. 25:710-713, 1995.

Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, et al.: A genome-wide search for human non insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nature Genetics, 13:161-166, 1996.

Hacia JG, Brody LC, Chee MS, Fodor SP, Collins FS: Detection of heterozygous mutations in BRCA1 using high density oligonucleotide arrays and two-colour fluorescence analysis. Nature Genetics. 14:367 370, 1996.

Heller RA, Schena M, Chai A, Shalon D, Bedilion T, Gilmore J, Woolley DE, Davis RW: Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. Proc Natl Acad Sci. USA. 94:2150-2155, 1997.

Hudson B, Stickland M, Futers S, and Grant P: Effects of novel polymorphisms in the RAGE gene on transcriptional regulation and their association with diabetic retinopathy. Diabetes 50: 1505-1511, 2001.

Jenkinson C, Wright L, Coulter A. Criterion validity and reliability of the SF-36 in a population sample. Quality of Life Research 3:7-12, 1994.

Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR: The changing natural history of nephropathy in type 1 diabetes. Am J Med. 78:785-794, 1985.

Krolewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradlely RF, Kahn CR: Magnitude and determinants of coronary artery disease in juvenile-onset, insulin dependent diabetes mellitus. Am J Cardiol. 59:750-755, 1987a.

Krolewski AS, Warram JH, Rand LI, Kahn CR: Epidemiologic approach to the etiology of diabetes mellitus and its complications. N Eng J Med. 317:1390-1398, 1987b.

Lunetta M, Le Moli R, Grasso G, Sangiorgio L. A simplified diagnostic test for ambulatory screening of peripheral diabetic neuropathy. Diabetes Research and Clinical Practice 39:165-72, 1998.

Maeda M, Yamamoto I, Fukuda M, et al: MTHFR gene polymorphism as a risk factor for diabetic retinopathy in Type 2 diabetic patients without serum creatinine elevation. Diabetes Care 26:547-548, 2003.

Marshall A, Hodgson J: DNA chips: An array of possibilities. Nature Biotecnhnology 16:27-31, 1998.

Measurement Excellence and Training Resource Information Center. Critical review of Michigan Neuropathy Screening Instrument (MNSI) and Michigan. Diabetic Neuropathy Score (MDNS). Available from URL:
http://www.measurementexperts.org//instrument/instrument_reviews.asp?detail=66.

Mogensen CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Eng J Med. 310:356-360, 1984.

Mogyorosi A and Ziyadeh FN: Update on pathogenesis markers and management of diabetic nephropathy. Cur Op in Nephrol and Hyperten. 5:243-253, 1996.

Munson PJ, Alizadeh A, Eisen M et al.: Second workshop on methods and applications of DNA microarray technology, Tuscon, Arizona, 1998.

Murata M, Maruyama T, Suzuki Y, Saruta T, and Ikeda Y : Paraoxonase 1 Gly/Arg polymorphism is associated with the risk of microangiopathy in Type 2 diabetes mellitus. Diabet. Med. 21: 837-844, 2004.

Nelson RG, Knowler WC, Pettitt DJ, Bennett MB: Kidney diseases in diabetes. in Diabetes in America. NIH/NIDDK, NIH Publication 95-1468, pp349-385, 1995.

Page R, Morris C, Williams J, von Ruhland C, Malik AN: Isolation of diabetes-associated kidney genes using differential display. Biochem Biophys Res Comm. 232:49-53, 1997.

Pettitt DJ, Saad MF, Bennett PH, Nelson RG, Knowler WC: Familial predisposition to renal disease in two generations of Pima Indians with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia. 33:438-443, 1990.

Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. Diabetologia. 39:940-945, 1996.

Ramsay G: DNA chips: state-of-the-art. Nature Biotechnology. 16:40-44, 1998.

Ray D, Mishra M, Ralph S, Read I, Davies R, and Brenchley P: Association of the VEGF gene with proliferative diabetic retinopathy but not proteinuria in diabetes. Diabetes 53: 861-864, 2004.

Rich S, Freedman BI, Bowden DW: Genetic epidemiology of diabetic complications. Diabetes Reviews. 5:165-173, 1997.

Ringquist S, Pecoraro C, Gilchrist CM, Styche A, Rudert WA, Benos PG, Trucco M: SOP^{3}v2: web-based selection of oligonucleotide primer trios for genotyping of human and mouse polymorphisms. Nucleic Acids Res. 2005;33(Web Server issue):W548-52, 2005.

Risch N: Linkage strategies for genetically complex traits. 1. Multilocus models. Am J Hum Genet. 46:222-228, 1990.

Risch N, Merikangas K: The future of genetic studies of complex human diseases. Science 273:1516-1517, 1996.

Risch N and Merikangas K. Genetic analysis of complex disease. Science 1997 275: 1329-1330.

Rogus JJ, Krolewski AS: Using discordant sib pairs to map loci for quantitative traits with high sibling recurrence risk. Am J Hum Genet. 59:1376-1381, 1996.

Rudofsky G. Jr., Reismann P, Witte S, Humpert P et al.: Asp299Gly and Thr399Ile genotypes of the TLF4 gene are associated with a reduced prevalence of diabetic neuropathy in patients with Type 2 diabetes. Diabetes Care 27: 179-183, 2004.

Sakane N, Yoshia T, Hoshioka, et al: Beta 3-adrenoreceptor gene polymorphism: a newly identified risk factor for proliferative retinopathy in NIDDM patients. Diabetes 46: 1633-1636, 1997.

Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. New Engl J Med. 320:1161-1165, 1989.

Schena M, Shalon D, Davis RW, Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science. 270: 467-470, 1995.

Schena M, Shalon D, Heller R et al.: Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. Proc Natl Acad Sci, USA. 93:10614-10619, 1996

Sharma K and Ziyadeh FN: Hyperglycemia and diabetic kidney disease. The case for transforming growth factor-b as a key mediator. Diabetes. 44:1139-1146, 1995.

Simon, R: Methods for the megavariate analysis of gene expression data. Second workshop on methods and applications of DNA microarray technology, Tuscon, Arizona, 1998.

Sivenius K, Pihlajamaki J, Partanen J, Niskanen L, Laakso M, and Uusitupa M: Aldose reductase gene polymorphisms and peripheral nerve function in patients with Type 2 diabetes. Diabetes Care 27: 2021-2026, 2004.

Southern EM, Maskos U & Elder JK:. Analyzing and comparing nucleic acid sequences by hybridization to arrays of oligonucleotides: evaluation using experimental models. Genomics. 13:1008-1017, 1992.

Southern EM: DNA chips: analysing sequence by hybridization to oligonucleotides on a large scale. Trends Genetics. 12:110-115, 1996

Spielman RS and Ewens WJ: The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet. 59:983-989, 1996.

Spielman RS and Ewens WJ: A sibship test for linkage in the presence of association: the S-TDT. Am J Hum Genet. 62:450-458, 1998.

Spielman RS, McGinnis RE, Ewens WJ: Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus. Am J Hum Genet. 52:506-516, 1993.

Striker GE, Peten EP, Carome MA, Pesce CM, Schmidt K, Yang C-W, Elliot SJ, Striker LJ: The kidney disease of diabetes mellitus (KDDM): a cell and molecular biology approach. Diabetes/Metabolism Reviews. 9:37-56, 1993.

Trevisan R and Viberti G: Genetic factors in the development of diabetic nephropathy. J Lab Clin Med. 126:342-349, 1995.

Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF III, Spielman RS, Dunaif A: Thirty-seven candidate genes for polycystic ovary syndrome: Strongest evidence for linkage is with follistatin. Proc Nat Acad Sci USA 96: 8573-8578, 1999.

Urbanek M, Wu X, Vickery, KR, Kao L-C, Christenson LK, Schneyer A, Legro RS, Driscoll DA, Strauss JF III, Dunaif A, Spielman RS: Allelic variants of the follistatin gene in polycystic ovary syndrome. J. Clin Endocr Metab 85:4455-4461, 2000.

Viberti GC, Keen H, Wiseman MJ: Raised arterial pressure in parents of proteinuric insulin dependent diabetes. Br Med J. 295:515-517, 1987.

Ware JJ, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Medical Care 30:473-83, 1992.

Warram JH, Gearin G, Laffel L, Krolewski AS: Effect of duration of type 1 diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinin ratio. J Am Soc Nephrol. 7:930-937, 1996.

Appendices

Appendix A: Candidate genes for Diabetic Complications (see legend)

EXTRACELLULAR MATRIX	SYMBOL	CHROMOSOME
collagen 4A1	COL4A1	13q34
collagen 4A2	COL4A2	13q35
collagen 4A3	COL4A3	2q36-q37
collagen 4A4	COL4A4	2q36-37
collagen 4A5	COL4A5	Xq22
collagen 4A6	COL4A6	Xq22
fibronectin 1	FN1	2q34
integrin, alpha 2	ITGA2	5q23-q31
integrin, alpha V	TGA5	12q11-q13
integrin, beta 1	ITGB1	10p11.2
laminin A4	LAMA4	6q21
laminin B1	LAMB1	7q22
laminin B2	LAMB2	3p21.1
nidogen (entactin)	NID	1q43
ENZYMES		
#aldose reductase	ALDR	7q35
*angiotensin converting enzyme	ACE	17q23
cathepsin B	CTSB	8p22
endothelin converting enzyme 1	ECE-1	1p36.1
metalloproteinase-3 (stromelysin)	MMP3	11q23
*methylenetetrahydrofolate reductase	MTHFR	1p36.2
*paraoxonase 1	PON1	7q21.1
protein kinase C, alpha	PRKCA	17q22-q23.2
protein kinase C, beta 1	PRKCB	16p11.2
renin	REN	1q32
tissue inhibitor of metalloproteinase 2	TIMP-2	17q25
tissue inhibitor of metalloproteinase 3	TIMP-3	22q12.1-q13.2
CYTOKINES & GROWTH FACTORS		
fibroblast growth factor 2 (basic)	FGF2	4q25-q27
insulin-like growth factor 1	IGF1	12q22-q24.1
insulin-like growth factor binding protein-1	IGFBP1	7p14-p12
platelet-derived growth factor beta	PDGFB	22q12.3-q13.1

transforming growth factor-beta1	TGFB1	19q13.1-q13.3
transforming growth factor-beta2	TGFB2	1q41
transforming growth factor-beta3	TGFB3	14q24
*vascular endothelial growth factor	VEGF	6p21.1

HORMONES

atrial natriuretic factor (peptide)	NPPA	1p32.6
adrenomedullin	M	11
angiotensinogen	AGT	1q42-q43
preproendothelin	EDN1	6p24-p23

RECEPTORS

AGE receptor	AGER	6p21.3
angiotensin-2 receptor 1A	AT2R1	3q21-q25
*beta-adrenergic receptor	ADRB2	5q31.1-qter
endothelin receptor A	EDNRA	12q22.1
endothelin receptor B	EDNRB	13q22
insulin-like growth factor 1 receptor	IGF1R	15q25-q26
insulin receptor-related receptor	INSRR	1q21-q22
PDGF receptor-beta	PDGFRB	5q31-q32
#Toll-like receptor 4	TLR4	
transforming growth factor-beta receptor II	TGFBR2	3p22
transforming growth factor-beta receptor III	TGFBR3	1p33-p32
#tumor necrosis factor receptor 4	TNFRSF1B	1p36

TRANSCRIPTION FACTORS

c-fos	FOS	14q24.3
c-jun	JUN	1p32-p31
c-myc	MYC	8q24.1-q24.13

OTHERS

apolipoprotein-E	APOE	19q13.2
glucose transporter-1; solute carrier family 2	GLUT1, SCL2A1	1p35-p31.3
Na+/H+ antiporter; solute carrier family 9	NHE1; SLC9A1	1p36.1-p35

Legend:

* Signifies candidate gene for retinopathy
# Signifies candidate gene for neuropathy
All others are candidate genes for nephropathy

Appendix B: Supporting Data

The information and new technology generated by the Human Genome Project are making it possible to perform large-scale, comprehensive, gene expression analyses. Technical advances in DNA microarray have made it possible to study hundreds to thousands of transcripts simultaneously. The identity and function of many transcripts are already available in public database such as dbEST and Unigene. Together, these advances should allow a different approach to studying the genetic basis of complex diseases. Instead of starting from genetic variation detected at the DNA level, and then determining whether that variation plays a role in gene expression and protein function, we can also study the gene expression pattern, then look for the genetic variation.