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ELMO1 variants and susceptibility to diabetic nephropathy in American Indians

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Abstract

Variants in the engulfment and cell motility 1 gene, *ELMO1,* have previously been associated with kidney disease attributed to type 2 diabetes. The Pima Indians of Arizona have high rates of diabetic nephropathy, which is strongly dependent on genetic determinants; thus, we sought to investigate the role of *ELMO1* polymorphisms in mediating susceptibility to this disease in this population. Genotype distributions were compared among 141 individuals with nephropathy and 416 individuals without heavy proteinuria in a family study of 257 sibships, and 107 cases with diabetic ESRD and 108 controls with long duration diabetes and no nephropathy. We sequenced 17.4 kb of *ELMO1* and identified 19 variants. We genotyped 12 markers, excluding those in 100% genotypic concordance with other variants or with a minor allele frequency <0.05, plus 21 additional markers showing association with ESRD in earlier studies. In the family study, the strongest evidence for association was with rs1345365 (odds ratio [OR]=2.42 per copy of A allele [1.35–4.32]; P=0.001) and rs10951509 (OR=2.42 per copy of A allele [1.31–4.48]; P=0.002), both of which are located in intron 13 and are in strong pairwise linkage disequilibrium (r^2=0.97). These associations were in the opposite direction from those observed in African Americans, which suggests that the relationship between diabetic kidney disease and *ELMO1* variation may involve as yet undiscovered functional variants or complex interactions with other biological variables.

Keywords

association analysis; candidate gene study; end stage renal disease; genetic susceptibility; Pima Indians

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1. Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in the United States and underlies a substantial proportion of morbidity and mortality associated with diabetes. Regardless of diabetes type, duration of the disease is one of the strongest determinants of diabetic nephropathy (1–3). In addition to duration of diabetes, other risk factors include hyperglycemia, hypertension, and hyperlipidemia (4; 5); however, strong evidence supports a role for genetic factors in mediating susceptibility to diabetic nephropathy and it is likely that the combination of environmental exposures and genetic load determine individual risk for development or progression of the disease (6–8).

Variants in the gene encoding the engulfment and cell motility 1 protein (*ELMO1*) are associated with kidney disease attributed to type 2 diabetes in Japanese (9) and African American individuals (10). Variants in *ELMO1* are also associated with kidney disease in European Americans with type 1 diabetes (11; 12). However, the particular nephropathy-associated variants have differed across populations, and the contribution of this gene to the development of diabetic nephropathy remains unclear. In the present study, we evaluated the role of *ELMO1* variants in diabetic nephropathy in American Indians. This population is at high risk for diabetic nephropathy, and the occurrence of the disease is strongly familial (13; 14). The goals of this study were twofold: 1) to identify additional *ELMO1* polymorphisms and test them for association with kidney disease in American Indian individuals with T2D, and 2) to assess in this population the association between diabetic kidney disease and *ELMO1* variants previously observed in other populations.

2. Materials and methods

2.1 Subjects

All subjects in this analysis are participants in a longitudinal study of type 2 diabetes and its complications conducted in the Gila River Indian Community since 1965 (15). In this study, community members were invited to biennial examinations, which included a 75 g oral glucose tolerance test, used to diagnose diabetes, and measurement of urinary protein-creatinine and (since 1982) albumin-creatinine ratio. Some of the individuals in the present analysis participated previously in a genome-wide linkage scan for diabetic nephropathy (13). A description of the study group in whom sequence variants were genotyped has also been published (16). Genotyping was performed in the family-based study of individuals who participated in the original linkage scan, and in a case-control study of diabetic ESRD. The family-based sample was comprised of 141 individuals with nephropathy (i.e., urinary protein-to-creatinine ratio >500mg/g) and 416 individuals without nephropathy (protein creatinine ratio < 500 mg/g) (13). Among the affected individuals 62% were women, the mean (± SD) age was 51.0 ± 11.5 years and mean duration of diabetes was 17.9 ± 7.9 years, while among the unaffected individuals 65% were women, the mean age was 42.2 ± 11.9 years and mean duration of diabetes was 8.0 ± 7.2 years. The case-control study consisted of 107 cases with diabetic ESRD (68% women, mean age=55.9 ± 8.9 years, mean duration=20.4 ± 7.1 years) and 108 control subjects with diabetes duration >10 years and a maximum urinary albumin-creatinine ratio <300 mg/g observed in the longitudinal study (56% women, mean age=58.8 ± 9.7 years, mean duration=20.7 ± 5.5 years); none of these

individuals was a first-degree relative of another in the sample. There were 71 individuals who were part of both the case-control and family-based studies. The study was approved by the Institutional Review Boards of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the Translational Genomics Research Institute. All subjects provided written informed consent prior to participation in the study.

2.2 SNP detection

We sequenced 17.4 kb of *ELMO1,* including all exons, exon-intron boundaries, and 3 kb of the 5′ regulatory region, in 36 Pima Indians (18 ESRD cases, 18 diabetic controls) to identify genetic variants. DNA was amplified in a final reaction volume of 10 μl using 60 ng genomic DNA, 10x standard PCR buffer containing 1.5 mM $MgCl_2$ (Applied Biosystems; Foster City, CA), 800 μM dNTPs, 0.4 μM oligonucleotide primers, and 0.4 U AmpliTaq Gold (Applied Biosystems). PCR cycling conditions consisted of an initial denaturation at 96°C for 7 min, followed by 35 cycles of 96°C for 20 s, 57°C for 30 s, and 72°C for 45 s, ending with a final elongation step at 72°C for 5 min. Following amplification, AMPure magnetic bead technology (Beckman Coulter Genomics; Danvers, MA) was used to remove unconsumed dNTPs and oligonucleotide primers. Amplicons were bidirectionally sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems; Foster City, CA) and 35 cycles of 96°C for 10 s, 55°C for 10 s, and 60°C for 4 m and subsequently purified with CleanSEQ magnetic bead technology (Beckman Coulter Genomics). Sequences were generated by the AB3730*xl* DNA Analyzer platform (Applied Biosystems) and analyzed using Mutation Surveyor software v2.61 (SoftGenetics; State College, PA). Information on all sequencing primers is available upon request.

In addition to variants identified by sequencing, we also selected SNPs that showed association with kidney disease in Japanese (9) and African American (10) individuals with T2D. These included 8 SNPs from the study conducted in Japanese individuals: rs1541727, rs4723596, rs11983698, rs4723593, rs7799004, rs1558688, rs3807163, and rs7804092 (9), and 13 SNPs from the study conducted in African American individuals: rs9969311, rs2717972, rs6462740, rs2058730, rs2160430, rs7782590, rs10951509, rs1981740, rs1345365, rs6979467, rs6462733, rs6967682, and rs6462731 (10).

2.3 SNP genotyping

SNPs were genotyped by either allelic discrimination PCR (AD-PCR) in conjunction with the 7000 Sequence Detection System (Applied Biosystems) or the iPLEX assay (Sequenom, Inc.; La Jolla, CA) according to the manufacturers' protocols. SNPs genotyped using AD-PCR included 37457413, rs1731981, rs1882072, rs1882071, rs13242348, rs2717968, rs17170970, rs1420421, 37139247, rs2072522, rs741301 and rs7785934. All remaining markers (i.e., rs1541727, rs9969311, rs2717972, rs6462740, rs2058730, rs2160430, rs7782590, rs10951509, rs1981740, rs1345365, rs6979467, rs6462733, rs6967682, rs6462731, rs4723596, rs11983698, rs4723593, rs7799004, rs1558688, rs3807163, rs7804092) were genotyped using the iPLEX platform. We designed iPLEX primers and multiplex conditions with the Assay Design v3.1 software (Sequenom). For the iPLEX assay, reaction products were dispensed onto a 384-element SpectroCHIP bioarray

(Sequenom) using a MassARRAY Nanodispenser, and analyzed by MassARRAY Workstation v4.0 software.

The observed genotype frequency for each SNP was assessed for deviation from that expected under Hardy-Weinberg equilibrium and encrypted samples were used to assess data quality. Assays were considered successful and genotype data subsequently analyzed if 1) a minimum of 90% of all genotyping calls were obtained, 2) markers did not deviate significantly ($P \leq 0.05$) from Hardy-Weinberg equilibrium, and 3) genotyping error results were <3%.

2.4 Power Calculations

Power for association studies depends on the sample size, the level of type I error specified, and the genotype frequencies in cases and controls. Genotype frequencies will depend on population frequency of the allele of interest and its effect on disease liability. For the present power calculations, a functional allele with an additive effect on disease liability was assumed (comprised of a mixture of three normal distributions). By 20 years of duration of diabetes, the cumulative incidence of ESRD in Pimas is ~0.15 and the cumulative incidence of macroalbuminuria is ~0.85, thus, the cases and controls are assumed to represent the upper and lower 15% of the liability distribution, respectively (17). Individuals in the family-based study were estimated by application of the generalized estimating equation approach to simulated data to represent an effective sample size of 133 cases and 395 controls for a dichotomous trait with prevalence of 24% (i.e., the upper 24% and the lower 76% of the liability distribution). With these assumptions, the minimal effect size detectable with 80% power at $p<0.05$ was calculated using the formulas derived by Hanson et al (18) for various values of the minor allele frequency (maf). The results, shown in Table 1, indicate that the present case-control study is well-powered to detect an association accounting for ~1.5% of the variance in liability; given the selection parameters this corresponds to an odd ratio of 1.7–3.8 per copy of the risk allele depending on its frequency. Similarly the family study is powered to detect an association accounting for ~2.7% of the variance, corresponding to odds ratios of 1.5–2.6.

2.5 Statistical Analysis

The extent to which observed genotype frequencies for each SNP deviated from that expected under Hardy-Weinberg equilibrium (HWE) was assessed (χ^2 with 1 df); none of the markers varied significantly from HWE. The statistical evidence for association and the strength of the association between genotype and affection status, as determined by the odds ratio (OR) and the corresponding 95% confidence interval (CI), were calculated by logistic regression. For these analyses, an “additive” model was used in which the genotype was coded as a numeric variable representing the number of risk alleles; thus, the OR designates the odds for nephropathy associated with each copy of the risk allele. In the family study, the logistic regression was performed using a non-linear “mixed” model (PROC NLMIXED in SAS) in which sibship was included as a random effect to account for the familial nature of the data; age, sex, duration of diabetes, and genotype were analyzed as fixed effects. In the case-control study, logistic regression was used without covariates (since duration of diabetes was included in the selection criteria). However, results were similar when age, sex

and duration of diabetes were included as covariates. To conduct a statistical test of association across both the case-control and family-studies, the p-values for each of the statistical tests were combined using Stouffer's method (19), which takes direction of association into account. To avoid duplication of individuals in the combined analysis, the p-value for the case-control study was re-calculated excluding individuals in the family study prior to conducting the combined test. Haplotype analyses were conducted as previously described (20).

Given the disparate findings across some populations, a meta-analysis of p-values from specific investigations of *ELMO1* variants [(10; 12; 21) and the present study] was conducted to assess the null hypothesis that the p-values are derived from the null distribution (no association with any variant). P-values were combined using the method of Brown, which is analogous to Fisher's method for combining p-values except that it accounts for correlation among SNPs (22; 23). The p-values were taken from published supplemental material where available (10; 12) or supplied by the authors of the original study (21). Correlations among SNPs (|r|) were estimated from the HapMap data.

3. Results

ELMO1 spans over 590 kb on 7p14.2 and contains 22 exons. We sequenced approximately 17.5 kb of the gene corresponding to all exons, exon-intron boundaries, and 3 kb of upstream regulatory sequence in 36 Pima Indian individuals (18 diabetic kidney disease cases and 18 diabetic controls). Using this approach, we identified 19 SNPs in the *ELMO1* locus (Fig. 1). Six SNPs (37457413, rs1731981, rs56005717, rs1882072, rs1882071, and rs13242348) were located in the promoter, and three were in coding regions, including rs2717968, 37238295, and rs13246439 in exons 6, 8, and 22, respectively. Five markers were not present in the public SNP database dbSNP (http://www.ncbi.nlm.nih.gov/SNP), and are designated here by genomic position (Build 36.1). Three pairs of markers showed complete genotypic concordance (i.e., rs2717969/rs2717968, 37277648/rs17170970, and rs2072522/rs2072521); only one SNP from each pair was genotyped in the study samples. Four markers (i.e., rs56005717, 37320773, 37238295, and rs13246439) had a minor allele frequency ≤0.05 and were not genotyped in the expanded study sample.

We genotyped 12 *ELMO1* SNPs (37457413, rs1731981, rs1882072, rs1882071, rs13242348, rs2717968, rs17170970, rs1420421, 37139247, rs2072522, rs741301, rs7785934). In addition, we selected 21 markers previously associated with diabetic kidney disease in earlier studies (9; 10). Linkage disequilibrium among the genotyped markers is shown in Figure 2. We evaluated association between the genotyped *ELMO1* markers and diabetic nephropathy as shown in Table 2. In the family study, the strongest evidence for association with diabetic nephropathy was found with markers rs10951509 (OR=2.42 per copy of the A allele [1.31–4.48]; P=0.002) and rs1345365 (OR=2.42 per copy of the A allele [1.35–4.32]; *P*=0.001). These two markers, located in intron 13, are within 13 kb of each other and are in strong pairwise linkage disequilibrium (r^2=0.97, D′=1.0). There was also evidence of association between diabetic nephropathy and 3 other SNPs in intron 13: rs7782590 (OR=2.13 [1.12–4.07]; P=0.0133), rs1981740 (OR=1.86 [1.03–3.38]; P=0.0319), rs6462733 (OR=2.21 [1.09–4.50]; P=0.0185). These markers were also in strong LD with

each other and with rs10951509 and rs1345365. Further, we observed a trend toward association with 3 additional markers in intron 13: rs6967682 (OR=1.97 [0.95–4.07]; P=0.0550), rs6462731 (OR=1.92 [0.96–3.85]; P=0.0530), and rs1420421 (OR=1.81 [0.94–3.50]; P=0.0663). However, none of these SNPs had a significant association ($p<0.05$) with nephropathy conditional on the association with rs1345365, although rs1345365 had a significant association conditional on each of these markers. Similarly, haplotype analyses showed that the most strongly associated haplotypes were those highly concordant with alleles at rs1345365 (supplemental figure). These observations suggest that the primary association in the family study is with rs1345365 (or rs1095109 which is statistically indistinguishable), and that the other associations are due to LD with these markers. However, rs1345365 was not significantly associated with ESRD in the case-control study, and neither were any of the other genotyped markers. When the family- and case-control studies were combined, the evidence for association was modest, and the strongest association was with rs1345365 (P=0.03). Results for the meta-analysis of the present study and previously published studies of *ELMO1* variants are shown in Table 3. The combined p-value across all variants for the present study was 0.2974, whereas across all studies combined, it was 0.1782.

4. Discussion

In this study, we screened *ELMO1*, including all exons, exon-intron boundaries, and the upstream regulatory region to identify variants that might increase susceptibility to diabetic nephropathy in Pima Indians. We identified 19 polymorphisms and genotyped 12 common, non-redundant SNPs ($r^2<1$), in conjunction with 21 *ELMO1* variants previously found to be associated with diabetic kidney disease in other populations.

We investigated *ELMO1* as a candidate gene for diabetic nephropathy based upon findings from two independent studies. The first was a genome-wide case-control study conducted to identify candidate genes for diabetic nephropathy in Japanese individuals (9). In that study, a SNP located on 7p14 showed the strongest association with diabetic nephropathy and subsequent linkage disequilibrium screening of a 200 kb region flanking the landmark SNP identified other markers showing association with the disease. Based on the absence of other genes within this linkage disequilibrium block, the authors concluded that *ELMO1* was the most likely candidate for diabetic nephropathy at this locus. In a subsequent study, different variants in this gene were associated with ESRD attributed to T2D in African American individuals (10). In addition, recent studies describe variants in *ELMO1* associated with diabetic nephropathy in type 1 diabetes (11; 12). However, a recent study in Mexican-Americans neither confirmed previous findings of association, nor observed significant associations with other *ELMO1* variants after correction for multiple comparisons (21). Previous studies in the Pimas identified linkage on chromosome 7, but this was 95 Mb away from *ELMO1*, and, thus, unlikely to reflect variation at this locus (13).

In the current study, we did not detect statistically significant evidence for association between diabetic nephropathy in Pima Indians and *ELMO1* variants associated with the disease in Japanese individuals with T2D (9). The SNPs associated with nephropathy in the current family study (i.e., rs1345365, rs1981740, and rs10951509) are the same as those

reported as associated in African American individuals with ESRD attributed to T2D (10), but the direction of association observed in Pimas was opposite to that in the African American study sample. For example, the A allele at marker rs1345365 was associated with increased risk of diabetic nephropathy in Pima Indians, whereas the same allele was associated with a lower risk of ESRD in African Americans. It is possible that disparate associations can occur spuriously, i.e., by chance alone. It is also possible, however, that different association patterns can occur between populations due to variable linkage disequlibrium patterns which result in functional variants occurring on different haplotypes (i.e., the “flip-flop” phenomenon (24)). It is noteworthy that LD extends for much greater distances in Pima Indians compared with African Americans. In addition, in Pimas, the A allele at rs1345365 is the major allele, whereas in African Americans, it is the minor allele. The same relationship is found among the other associated intron 13 markers, with the exception of rs2058730, in which the A allele is the major allele in both populations. It is also worth noting that, while associations between *ELMO1* variants and nephropathy have been observed in many populations, the specific allelic associations have not replicated across populations. Different variants are associated with risk of diabetic nephropathy in Japanese, African Americans, European Americans (9–12) and, now, American Indians. Such a pattern of disparate associations within a gene might be expected if numerous rare functional variants influenced the risk of diabetic nephropathy. Investigation of these markers in other populations, along with resequencing studies, may help to resolve the disparate findings of association between *ELMO1* variants and diabetic kidney disease.

The role of chance in producing the disparate association patterns is difficult to exclude without clear replication in an independent group of individuals. In this respect, the lack of replication between the family and case-control studies suggests that the associations observed in the former may be spurious. Yet it is also possible that biological differences in the affection status could result in lack of comparability across the two studies. In the family study, nephropathy was defined on the basis of proteinuria, while in the case-control study it was defined on the basis of ESRD. Genetic factors that influence initiation of diabetic nephropathy in the form of proteinuria may be different from those that influence progression to ESRD (25). In such a situation, the power for replication may be diminished.

When the current family and case-control studies were combined, the associations between *ELMO1* variants and diabetic kidney disease remained nominally significant. *ELMO1* is a large gene and in the present study, as in most others in the literature, a large number of variants were tested for association with nephropathy. In such a situation, the potential for chance findings arising on account of multiple comparisons should be considered. In fact, results from the meta-analysis presented here suggest that the distribution of p-values across the published studies is consistent with the null hypothesis of no true associations, although the power of this analysis is likely quite low. Correction for multiple comparisons in genetic studies should take into account the fact that genotyped variants within a gene are not independent. A simple Bonferroni correction for the number of genotyped variants is too stringent, as it ignores the dependence among SNPs. One simple method to account for multiple comparisons is to correct for the effective number of variants, estimated from the number of haplotype blocks plus the number of SNPs outside of blocks (26). This method would imply eight effective tests for the present study, which does not yield a statistically

significant result in the combined analysis (although the results for the family study remain significant).

With the present sample sizes, power is low to detect variants with modest effects on susceptibility to diabetic nephropathy. Based on the cumulative incidence of ESRD and heavy proteinuria in this population (13; 14), we estimate that the smallest odds ratio detectable at $p<0.05$ with 80% power for a risk allele with frequency of 0.2 is 1.7 for the family study and 2.0 for the case-control study (18), and these are stronger effects than those reported previously for many *ELMO1* variants (ORs ~1.3). It is noteworthy, however, that for many of the SNPs previously associated with nephropathy, the associations in the current study are in the opposite direction of the original report, and the confidence intervals reported here exclude the previously-reported odds ratios. For example, in African Americans the odds ratio was 0.75 per copy of the A allele for rs1345365 (10), and in Japanese subjects the odds ratio was 0.75 per copy of the T allele at rs7799004 (9). The present study did not attempt a thorough examination of all common variation in *ELMO1*; instead we chose to focus on variants that were more likely to be functional based on proximity to coding regions and/or association in previous studies. Thus, it is possible that associations were missed for variants that were not well-captured with this approach.

In summary, we have undertaken a systematic analysis of the *ELMO1* locus in this study to identify genetic variants associated with kidney disease attributed to T2D in Pima Indians. These results identify novel *ELMO1* variants and nominally significant associations with heavy proteinuria for markers in intron 13 of this gene. However, the associations did not replicate in a second sample of cases and controls ascertained for ESRD and were in the opposite direction of those previously reported in African Americans. Further genotyping of these and other variants in diverse populations is necessary to determine what role, if any, *ELMO1* variants play in susceptibility to diabetic nephropathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Nelson RG, Kunzelman CL, Pettitt DJ, Saad MF, Bennett PH, Knowler WC: Albuminuria in type 2 (non-insulin-dependent) diabetes mellitus and impaired glucose tolerance in Pima Indians. Diabetologia 32:870–876, 1989 [PubMed: 2612758]
2. Pugh JA, Medina R, Ramirez M: Comparison of the course to end-stage renal disease of type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic nephropathy. Diabetologia 36:1094–1098, 1993 [PubMed: 8243860]
3. Gall MA, Rossing P, Skott P, Damsbo P, Vaag A, Bech K, Dejgaard A, Lauritzen M, Lauritzen E, Hougaard P, et al.: Prevalence of micro- and macroalbuminuria, arterial hypertension, retinopathy

and large vessel disease in European type 2 (non-insulin-dependent) diabetic patients. Diabetologia 34:655–661, 1991 [PubMed: 1955098]

4. Nelson RG, Knowler WC, Pettitt DJ, Hanson RL, Bennett PH: Incidence and determinants of elevated urinary albumin excretion in Pima Indians with NIDDM. Diabetes Care 18:182–187, 1995 [PubMed: 7729295]
5. Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R: Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. Arch Intern Med 158:998–1004, 1998 [PubMed: 9588433]
6. 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 [PubMed: 2401399]
7. 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 [PubMed: 8858216]
8. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 320:1161–1165, 1989 [PubMed: 2710189]
9. Shimazaki A, Kawamura Y, Kanazawa A, Sekine A, Saito S, Tsunoda T, Koya D, Babazono T, Tanaka Y, Matsuda M, Kawai K, Iizumi T, Imanishi M, Shinosaki T, Yanagimoto T, Ikeda M, Omachi S, Kashiwagi A, Kaku K, Iwamoto Y, Kawamori R, Kikkawa R, Nakajima M, Nakamura Y, Maeda S: Genetic Variations in the Gene Encoding ELMO1 Are Associated With Susceptibility to Diabetic Nephropathy. Diabetes 54:1171–1178, 2005 [PubMed: 15793258]
10. Leak TS, Perlegas PS, Smith SG, Keene KL, Hicks PJ, Langefeld CD, Mychaleckyj JC, Rich SS, Kirk JK, Freedman BI, Bowden DW, Sale MM: Variants in intron 13 of the ELMO1 gene are associated with diabetic nephropathy in African Americans. Ann Hum Genet 73:152–159, 2009 [PubMed: 19183347]
11. Craig DW, Millis MP, DiStefano JK: Genome-wide SNP genotyping study using pooled DNA to identify candidate markers mediating susceptibility to end-stage renal disease attributed to Type 1 diabetes. Diabet Med 26:1090–1098, 2009 [PubMed: 19929986]
12. Pezzolesi MG, Katavetin P, Kure M, Poznik GD, Skupien J, Mychaleckyj JC, Rich SS, Warram JH, Krolewski AS: Confirmation of genetic associations at ELMO1 in the GoKinD collection supports its role as a susceptibility gene in diabetic nephropathy. Diabetes 58:2698–2702, 2009 [PubMed: 19651817]
13. Imperatore G, Knowler WC, Nelson RG, Hanson RL: Genetics of diabetic nephropathy in the Pima Indians. Curr Diab Rep 1:275–281, 2001 [PubMed: 12643210]
14. Nelson RG, Newman JM, Knowler WC, Sievers ML, Kunzelman CL, Pettitt DJ, Moffett CD, Teutsch SM, Bennett PH: Incidence of end-stage renal disease in type 2 (non-insulin-dependent) diabetes mellitus in Pima Indians. Diabetologia 31:730–736, 1988 [PubMed: 3240833]
15. Knowler WC, Bennett PH, Hamman RF, Miller M: Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. Am J Epidemiol 108:497–505, 1978 [PubMed: 736028]
16. Wolford JK, Yeatts KA, Red Eagle AR, Nelson RG, Knowler WC, Hanson RL: Variants in the gene encoding aldose reductase (AKR1B1) and diabetic nephropathy in American Indians. Diabet Med 23:367–376, 2006 [PubMed: 16620264]
17. Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Bennett PH, Hanson RL: Segregation analysis of diabetic nephropathy in Pima Indians. Diabetes 49:1049–1056, 2000 [PubMed: 10866059]
18. Hanson RL, Looker HC, Ma L, Muller YL, Baier LJ, Knowler WC: Design and analysis of genetic association studies to finely map a locus identified by linkage analysis: sample size and power calculations. Ann Hum Genet 70:332–349, 2006 [PubMed: 16674556]
19. Stouffer SA, Suchman EA, DaVinney LC, Star SA, Williams RM : How the volumes were produced In The American Soldier, Volume i: Adjustment to Army Life Princeton, NJ, Princeton University Press, 1949, p. 45
20. Vozarova de Courten B, Hanson RL, Funuhashi T, Lindsay RS, Matsuzawa Y, Tanaka S, Thameem F, Gruber JD, Froguel P, Wolford JK. Common polymorphisms in the adiponectin gene *ACDC* are not associated with diabetes in Pima Indians. Diabetes. 54:284–289, 2005 [PubMed: 15616040]

21. Kim S, Abboud HE, Pahl MV, Tayek J, Snyder S, Tamkin J, Alcorn H Jr., Ipp E, Nast CC, Elston RC, Iyengar SK, Adler SG: Examination of Association with Candidate Genes for Diabetic Nephropathy in a Mexican American Population. Clin J Am Soc Nephrol. 5:1072–1078, 2010 [PubMed: 20299368]

22. Brown M: A method for combining non-independent one-sided tests of significance. Biometrics 31:987–992, 1975

23. Elston RC: On Fisher's method of combining p-values. Biometrical J 3:339–345, 1991

24. Lin PI, Vance JM, Pericak-Vance MA, Martin ER: No gene is an island: the flip-flop phenomenon. Am J Hum Genet 80:531–538, 2007 [PubMed: 17273975]

25. Placha G, Canani LH, Warram JH, Krolewski AS: Evidence for different susceptibility genes for proteinuria and ESRD in type 2 diabetes. Adv Chronic Kidney Dis 12:155–169, 2005 [PubMed: 15822051]

26. Duggal P, Gillanders EM, Holmes TN, Bailey-Wilson JE: Establishing an adjusted p-value threshold to control the family-wide type 1 error in genome wide association studies. BMC Genomics 9:516, 2008 [PubMed: 18976480]

27. Ke X, Hunt S, Tapper W, Lawrence R, Stavrides G, Ghori J, Whittaker P, Collins A, Morris AP, Bentley D, Cardon LR, Deloukas P: The impact of SNP density on fine-scale patterns of linkage disequilibrium. Hum Mol Genet 13:577–588, 2004 [PubMed: 14734624]

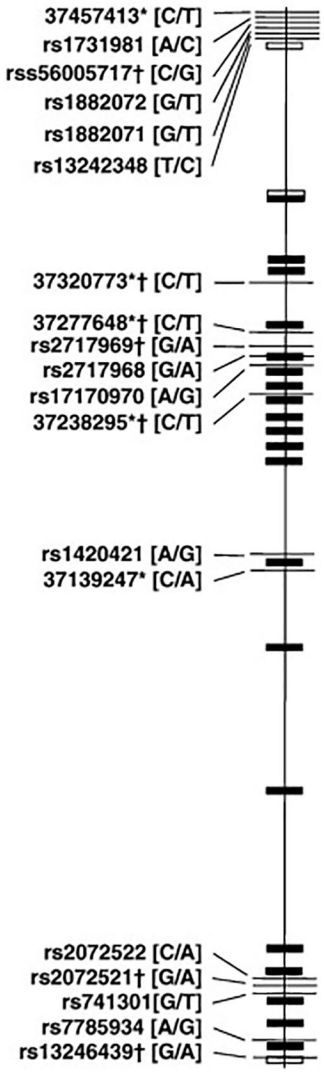

Figure 1. Location of SNPs identified by sequencing in the *ELMO1* locus.

Vertical lines represent the 19 markers identified by sequencing in Pima Indian individuals with T2D with (N=18) or without (N=18) kidney disease. Marker names are given below the gene with the alleles shown in brackets. Exons are designated as rectangles and are numbered consecutively, although the gene is transcribed in the reverse orientation; unfilled exons are untranslated, while filled exons are coding regions. Asterisked markers are novel SNPs not yet present in public databases and are named after chromosomal position (NCBI Build 36.1).

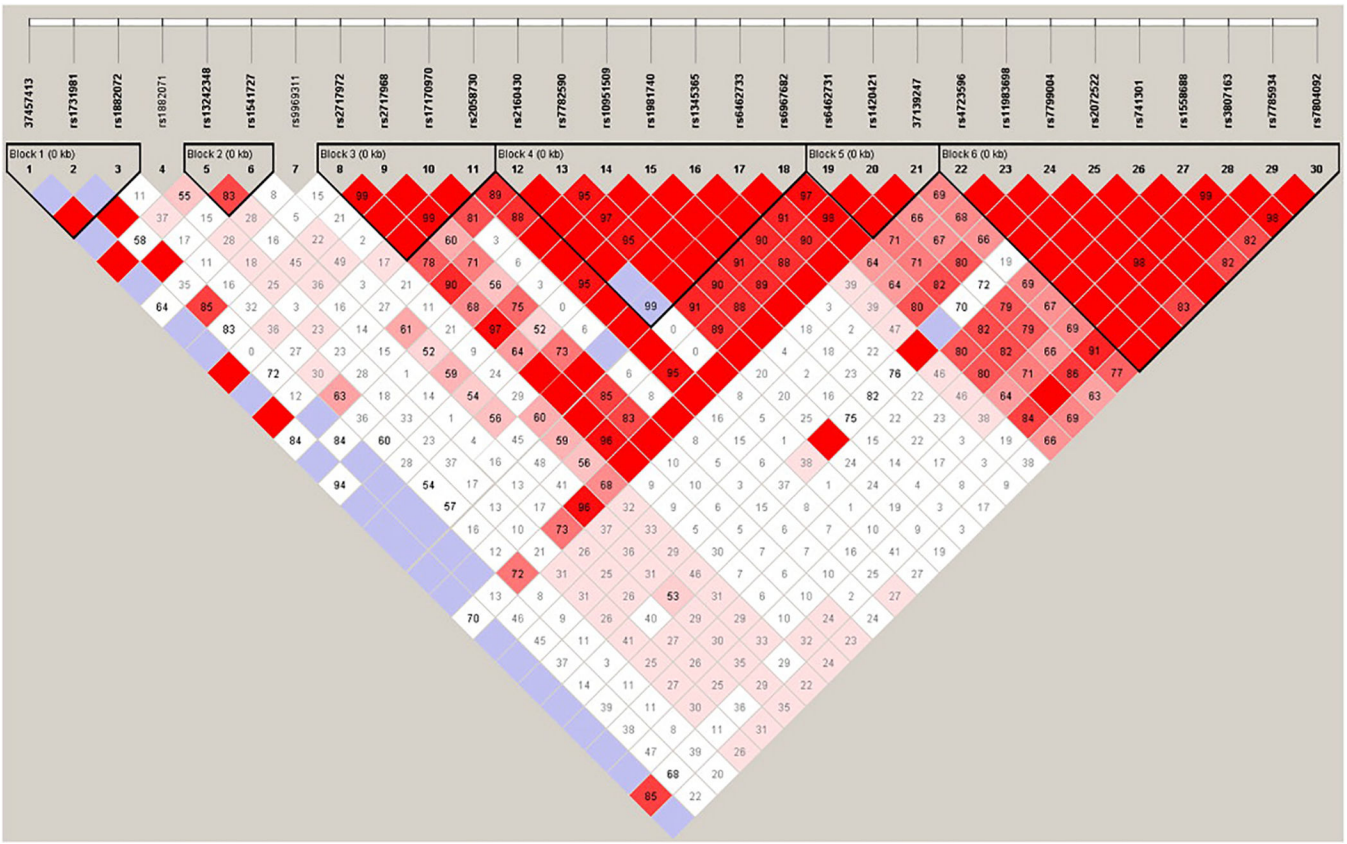

Figure 2. Pairwise linkage disquilibrium and haplotype block structure for *ELMO1* variants.

Gene structure and relative SNP position are shown at top of figure; marker name and orientation is designated below. For these analyses, a single individual from each family was selected. Haplotype blocks were estimated using the solid 'spine' method with D'>0.8 taken as the criterion to extend the spine (27). LD is represented in this figure as a measure of D', which is shown within each diamond. Darker shading designates strong LD, while lighter shading designates weak LD.

Table 1.

Minimal detectable effect size at P<0.05 with 80% power for case-control and family association studies.

	Case-Control Study		Family Study	
Maf	**OR**	**h^2**	**OR**	**h^2**
0.05	3.80	0.017	2.57	0.030
0.10	2.54	0.016	1.96	0.029
0.20	1.99	0.015	1.65	0.028
0.30	1.82	0.015	1.55	0.027
0.40	1.75	0.015	1.50	0.027
0.50	1.73	0.015	1.49	0.027

OR represents the odds ratio per copy of the risk allele and h^2 is the proportion of variance in liability to disease explained by the variant.

Table 2.
Association of ELMO1 genotypes with diabetic nephropathy.

Fam_OR and fam_p_val are family-based odds ratio and p-value, respectively. CC_OR and CC_p_val are case-control odds ratio and p-value. Comb_p_val is combined p-value

Marker	Pos	Fam_OR	fam_p_val	CC_OR	CC_p_pval	Comb_p_val
rs1882072	37456528	**1.16**	0.5344	0.89	0.6345	0.5903
rs1882071	37456113	0.88	0.5472	1.07	0.7536	0.9255
rs13242348	37455513	1.03	0.8974	1.06	0.7608	0.6475
rs1541727	37448950	0.82	0.3384	1.01	0.9469	0.8676
rs9969311	37381582	0.95	0.8151	1.08	0.6979	0.9728
rs2717972	37270120	0.92	0.6947	1.02	0.9247	0.8214
rs2717968	37265325	1.16	0.5051	0.83	0.3938	0.6316
rs17170970	37265247	0.89	0.699	1.16	0.6253	0.5859
rs2058730	37201281	0.87	0.5039	0.9	0.6161	0.6435
rs2160430	37189926	0.74	0.2379	1.17	0.5842	0.1483
rs7782590	37182849	2.13	0.0133	0.89	0.6503	0.0945
rs10951509	37180008	2.42	0.0022	0.95	0.8579	0.0487
rs1981740	37178829	1.86	0.0319	0.87	0.621	0.1525
rs1345365	37167138	2.42	0.0013	1	0.9957	0.0333
rs6462733	37156909	2.21	0.0185	0.84	0.5898	0.0972
rs6967682	37156400	1.97	0.055	0.72	0.3615	0.226
rs6462731	37154082	1.92	0.053	0.86	0.6603	0.1316
rs1420421	37139573	1.81	0.0663	0.76	0.4065	0.2048
37139247	37139247	0.86	0.5956	0.84	0.5376	0.5019
rs4723596	36917569	1.05	0.7931	1.21	0.3433	0.4743
rs11983698	36915072	1.16	0.466	1.16	0.4415	0.3419
rs7799004	36895489	1.21	0.3354	1.22	0.2955	0.1836
rs2072522	36893603	0.56	0.0432	1.06	0.8518	0.2545
rs741301	36884520	1.2	0.3518	1.2	0.3352	0.1647
rs1558688	36881710	1.11	0.5906	1.29	0.1917	0.2346
rs3807163	36875284	1.08	0.7016	1.21	0.3369	0.4233
rs7785934	36868104	0.92	0.7227	0.87	0.536	0.6211
rs7804092	36859757	1.02	0.9238	1.19	0.3702	0.6615

Table 3.

Meta-analysis of p-values across studies of ELMO1 variants and diabetic nephropathy.

Population	Reference	N Markers	Σ−2ln(p)	Corrected χ^2	Effective df	p-value
African Americans, Type 2 diabetes	[10]	88	279.3	36.2	22.8	0.0375
European Americans, Type 1 diabetes	[12]	118	241.0	20.7	20.3	0.4317
Mexican Americans, Type 2 diabetes	[21]	46	81.6	38.6	43.6	0.6830
American Indians, Type 2 diabetes	Present	28	66.8	10.0	8.4	0.2974
Combined			11.4	11.4	8.0	0.1782

The method of Brown (22) was used to combine p-values for all SNPs across ELMO1 in each study, with accounting for the correlation among SNPs. Σ−2ln(p) represents the sum of the twice the negative of the logarithm of the p-value across all SNPs. The corrected χ^2 and effective df are calculated from the correlation among SNPs (estimated from HapMap for all previous studies and from the current data in the present study). The combined value across all studies is calculated by Fisher's method (23).