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Confirmation of Genetic Associations at ELMO1 in the GoKinD Collection Supports Its Role as a Susceptibility Gene in Diabetic Nephropathy

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OBJECTIVE—To examine the association between single nucleotide polymorphisms (SNPs) in the engulfment and cell motility 1 (ELMO1) gene, a locus previously shown to be associated with diabetic nephropathy in two ethnically distinct type 2 diabetic populations, and the risk of nephropathy in type 1 diabetes.

RESEARCH DESIGN AND METHODS—Genotypic data from a genome-wide association scan (GWAS) of the Genetics of Kidneys in Diabetics (GoKinD) study collection were analyzed for associations across the ELMO1 locus. In total, genetic associations were assessed using 118 SNPs and 1,705 individuals of European ancestry across the ELMO1 locus. In total, genetic associations were assessed using 118 SNPs and 1,705 individuals of European ancestry with type 1 diabetes (885 normoalbuminuric control subjects and 820 advanced diabetic nephropathy case subjects).

RESULTS—The strongest associations in ELMO1 occurred at rs11769038 (odds ratio [OR] 1.24; 95% CI 1.15–1.34) and rs1882080 (OR 1.23; 95% CI 1.14–1.34) located in intron 16. Two additional SNPs, located in introns 18 and 20, respectively, were also associated with diabetic nephropathy. No evidence of association for variants previously reported in type 2 diabetes was observed in our collection.

CONCLUSIONS—Using GWAS data from the GoKinD collection, we comprehensively examined evidence of association across the ELMO1 locus. Our investigation marks the third report of associations in ELMO1 with diabetic nephropathy, further establishing its role in the susceptibility of this disease. There is evidence of allelic heterogeneity, contributed by the diverse genetic backgrounds of the different ethnic groups examined. Further investigation of SNPs at this locus is necessary to fully understand the commonality of these associations and the mechanism(s) underlying their role in diabetic nephropathy.

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Diabetic nephropathy is a major late complication of diabetes that affects ~30–40% of all patients with either type 1 or type 2 diabetes and continues to be the leading contributor to end-stage renal disease (ESRD) in the U.S. (1–3). In both type 1 and type 2 diabetes, diabetic nephropathy has been shown to cluster in families (4–8). Despite its known familial aggregation and intense effort to determine the genetic components that underlie its risk, including both candidate gene investigations and genome-wide linkage scans, no major gene that contributes to its susceptibility has yet been identified (9).

Variants in the engulfment and cell motility 1 (ELMO1) gene, located on chromosome 7p, have previously been shown to be associated with diabetic nephropathy in Japanese patients with type 2 diabetes (10). Subsequent functional studies demonstrated increased expression of ELMO1 in the presence of high glucose. In support of a potential role in the pathogenesis of diabetic nephropathy, overexpression of ELMO1 inhibited cell adhesion while promoting excess transcription growth factor-β, collagen type I, fibronectin, and integrin-linked kinase expression (10,11). Linkage of ESRD in type 2 diabetes has been shown with the 7p region in African Americans (12). Strong support for linkage with variation in glomerular filtration rate has also been reported at this same region in Caucasians (13). Leak et al. (14) recently examined genetic variants across ELMO1 in two large African American cohorts with type 2 diabetes and ESRD, and, in support of its potential role in the susceptibility of diabetic nephropathy, variants in intron 13 were found to be associated with disease.

We recently performed a genome-wide association scan (GWAS) for diabetic nephropathy susceptibility genes in type 1 diabetes and reported the identification of several novel susceptibility loci from the initial analysis of these data (15). In addition to uncovering associations at novel loci across the genome, these data also allow for the comprehensive examination of specific candidate disease loci. In this report, we investigated the role of 118 variations in ELMO1 on the risk of diabetic nephropathy in 1,705 Caucasian patients with type 1 diabetes using genotypic data from this GWAS.

RESEARCH DESIGN AND METHODS
A detailed description of the Genetics of Kidneys in Diabetes (GoKinD) study collection has been published previously (16). Briefly, subjects for the GoKinD collection were recruited through two centers: the George Washington University (GWU) Biostatistics Center and the Section of Genetics and Epidemiology at the Joslin Diabetes Center (JDC). Subjects enrolled in GoKinD by either recruitment center had type 1 diabetes diagnosed before age 31 years, began insulin treatment within 1 year of diagnosis, and were between 18 and 59 years of age at the time of enrollment. Case subjects with advanced diabetic nephropathy had either persistent proteinuria, defined by a urinary albumin-to-creatinine ratio (ACR) ≥300 μg/mg in two of the last three measurements taken at least 1 month apart, or ESRD (dialysis or renal transplant). Control subjects had type 1 diabetes for at least 15 years and normoalbuminuria, defined by an ACR <20 μg/mg in two of the last three measurements taken at least 1 month apart (if a third measurement was required, a value <40 μg/mg was necessary for inclusion), without ever having
TABLE 1
Baseline clinical characteristics of the GoKinD collection

<table>
<thead>
<tr>
<th></th>
<th>GoKinD collection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control subjects</td>
</tr>
<tr>
<td>n</td>
<td>885</td>
</tr>
<tr>
<td>Men/women</td>
<td>363/522</td>
</tr>
<tr>
<td>Age at type 1 diabetes diagnosis (years)</td>
<td>12.9 ± 7.4</td>
</tr>
<tr>
<td>Duration of type 1 diabetes (years)*</td>
<td>25.4 ± 7.8</td>
</tr>
<tr>
<td>Age at examination (years)</td>
<td>38.3 ± 8.7</td>
</tr>
<tr>
<td>Laser treatment (%)</td>
<td>17</td>
</tr>
<tr>
<td>A1C (%)†</td>
<td>7.4 ± 1.2</td>
</tr>
<tr>
<td>ACR (µg/mg)†</td>
<td>6.5 ± 3.7</td>
</tr>
<tr>
<td>Proteinuric subjects</td>
<td>1,520 ± 1,478</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>117.6 ± 11.9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71.4 ± 7.8</td>
</tr>
<tr>
<td>Case subjects with proteinuria/ESRD</td>
<td>—</td>
</tr>
<tr>
<td>ESRD duration (years)</td>
<td>—</td>
</tr>
<tr>
<td>Kidney transplant (%)‡</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ±SD unless otherwise indicated. *The duration of type 1 diabetes in control and proteinuric subjects is based on the duration of type 1 diabetes at the onset of ESRD. All other clinical characteristics are based on measurements performed at examination. †Mean A1C values do not include data from case subjects that have undergone pancreas transplantation (32%). ‡Percentages are of ESRD group.

been treated with ACE inhibitors or angiotensin receptor blockers and not being treated with antihypertensive medication at the time of recruitment into the study. A total of 1,705 individuals (885 control subjects and 820 advanced diabetic nephropathy case subjects, including 284 with proteinuria and 536 with ESRD) of European ancestry (confirmed by population ancestry and diabetic nephropathy case subjects, including 284 with proteinuria and 536 with ESRD) of European ancestry (confirmed by population ancestry and substructure analysis using EIGENSOFT; for further details see Pezzolesi et al. [15]) were included in the current study (Table 1).

Genotyping and imputation. The GoKinD collection was genotyped on the Affymetrix 5.0 500K SNP Array, a genotyping platform with estimated single-nucleotide polymorphism (SNP) efficiency of 0.98 and multi-marker genomic coverage of 76 and 84%, respectively, by the Genetic Association Information Network (GAIN) genotyping laboratory at the Eli and Edythe L. Broad Institute (Cambridge, MA) (17). After internal quality control, the GAIN genotyping laboratory released genotypes for 467,144 single nucleotide polymorphisms (SNPs). Details of the quality control criteria applied to these data have been published previously (15). Briefly, the application of quality control metrics for minor allele frequency (MAF) <0.01, rejection of Hardy-Weinberg assumptions (P < 10^{-5}), and differential rates of missing data (by case/control subject status) resulted in high-quality genotypic data for 359,193 autosomal SNPs.

Genotypic association with diabetic nephropathy for all 118 SNPs in ELMO1 under an additive genetic model is shown in Fig. 1 (also see supplemental Table S1 in the online appendix, available at http://diabetes.diabetesjournals.org/content/early/2009/08/02/db09-0641/suppl/DC1). A total of eight SNPs showed nominal evidence of association with diabetic nephropathy (P < 0.05) among the 885 control and 820 case subjects (Table 2). The strongest associations occurred at rs1769038 (OR 1.24; P = 1.7 × 10^{-5}) and rs1882080 (OR 1.29; P = 3.2 × 10^{-5}). These two SNPs map to intron 16 (located ≈12.5 kb apart) and are in near-complete LD (r^2 = 0.98). Two additional SNPs, rs2041801 (intron 18) and rs7785934 (intron 20), were also associated with diabetic nephropathy in the GoKinD samples (OR 1.22, P = 5.6 ×
The most strongly associated SNPs (Table 2) were rs7785934 and either rs11769038 or rs1882080 (P = 1.3 x 10^-5 and P = 1.7 x 10^-7, respectively). Although rs2041801 is in tight LD with rs11769038 and rs1882080 (r^2 = 0.97), LD among these three variants and rs7785934 is diminished (r^2 = 0.60–0.62). The four associated SNPs localize to three distinct LD blocks. Haplotype analysis of these three blocks (using representative tagging SNPs) did not yield more significant differences between case and control subjects than our single SNP analysis (data not shown). Two-SNP haplotype analysis of rs7785934 in combination with either rs11769038 (P = 2.5 x 10^-5) or rs1882080 (P = 3.7 x 10^-5) yielded more significant differences between case and control subjects than the single-SNP analysis of these variants.

Because recent studies have suggested that the development of proteinuria and its progression to ESRD may be influenced by different genetic factors, we analyzed the eight nominally significant ELMO1 SNPs for association with prevalent proteinuria (n = 284) and ESRD (n = 536) (Table 2) (20, 21). Only SNP rs7785934 was found to be primarily associated with ESRD (OR 1.33, P = 3.3 x 10^-4 vs. OR 1.03, P = 0.77 in proteinuria). Additionally, analysis of the two-SNP haplotypes formed by rs7785934 and either rs11769038 or rs1882080 was more strongly associated with ESRD than the individual SNPs (P = 1.3 x 10^-5 and P = 1.7 x 10^-7, respectively), whereas no association of these haplotypes was observed in patients with proteinuria (P = 0.36 and P = 0.43, respectively). We also examined the effects of this exposure on the SNPs (Table 2) by stratifying case and control subjects across tertiles of diabetes duration (at the onset of ESRD or at enrollment into GoKinD for proteinuric and control subjects) (22). The strength of the associations was consistent across these strata (data not shown). Finally, we investigated whether differences in HbA1c levels between case and control subjects contributed to the associations at these SNPs. No evidence of interaction was observed with this quantitative trait (P = 0.18–0.98).

The ELMO1 variants previously shown to be associated with diabetic nephropathy in type 2 diabetes were examined for evidence of association under additive, dominant, and recessive genetic models (Table 3). Three variants that had been reported to be associated with diabetic nephropathy in Japanese case subjects with type 2 diabetes (rs1558688, rs741301, and rs7785934) achieved nominal significance in the GoKinD collection (P ≤ 0.03); however, the SNP genotypes had an opposite direction of genetic effect in this collection and, thus, did not confirm the previous associations (supplemental Table S2 in the online appendix). The allele frequencies of these three variants in both the GoKinD and CEU HapMap (www.hapmap.org) populations are similar (data not shown).

**DISCUSSION**

Genetic variants in ELMO1 have recently been shown to be associated with diabetic nephropathy in two independent and ethnically distinct collections of patients with type 2 diabetes (10, 14). In this report, we examined whether variants in this same gene are associated with the risk of diabetic nephropathy in patients with type 1 diabetes. Through our comprehensive analysis of this locus, we extend these previous findings by demonstrating that variants in ELMO1 are also associated with the risk of diabetic nephropathy in Caucasian type 1 diabetic patients.

We investigated the role of 118 SNPs in ELMO1, including 12 SNPs previously reported to be associated...
with diabetic nephropathy in type 2 diabetes. Our analysis identified associations at several intronic SNPs (rs7785934, rs2041801, rs11769038, and rs1882080; P = 1.7 × 10⁻³ to 5.6 × 10⁻⁵). Although none of these SNPs met stringent criteria for significance following adjustment for multiple testing (P < 0.05/118 = 4.3 × 10⁻⁴), this threshold was exceeded by one SNP (rs7785934) when our analysis was limited to case subjects with ESRD. Moreover, the modest effect size of this variant (OR 1.33) is consistent with those previously reported in two independent African American ESRD populations (14), suggesting a comparable effect in the two populations. Additionally, the two-SNP haplotypes formed by rs7785934 and either rs11769038 or rs1882080 were more strongly associated with ESRD than these individual SNPs, suggesting that the LD block containing these SNPs forms a larger haplotype that either contains or is in tight LD with the causal variant at this locus. Together, these data also suggest that ELMO1 may have a role in the advanced stages of diabetic nephropathy, perhaps contributing to renal function decline, rather than its initiation. We acknowledge, however, that the GoKinD collection is heavily weighted with case subjects with ESRD. The small number of case subjects with proteinuria may have limited our ability to detect ELMO1 variants that are primarily associated with the risk of proteinuria. Despite this limitation, functional studies have demonstrated that ELMO1 contributes to the progression of chronic glomerular injury through its dysregulation of extracellular matrix (ECM) metabolism, resulting in renal ECM accumulation (11). This accumulation contributes to both glomerular and tubular basement membrane thickening, two well-established hallmarks of advanced diabetic nephropathy (23).

Our investigation marks the third report of genetic associations in ELMO1 with diabetic nephropathy, further establishing its role in conferring increased susceptibility to this disease. Previous reports (10,14) identified their strongest associations at variants located more than 280 kb apart in introns 17 and 13. Although our strongest associations are located near the associated SNP reported by Shimazaki et al. (10), our most associated SNPs are independent of those reported in this study. Furthermore, no evidence of association for the variants reported in either type 2 diabetic population was identified in our collection. The associations at ELMO1 across each study are consistent with allelic heterogeneity, likely contributed by the diverse ancestral genetic backgrounds of the different ethnic groups. Examination of the associated SNPs from each study in the available HapMap populations (www.hapmap.org) confirms the variable allele frequencies of these variants among different ethnic and racial groups (data not shown). We hypothesize that rare polymorphisms in ELMO1, either the same variants or those in strong or complete LD, may be common to each ethnic group and merely tagged by the common variants identified in each study. Further investigation of rare SNPs at the ELMO1 locus is necessary to fully understand the commonality of these associations and to elucidate the mechanism(s) underlying their role in diabetic nephropathy.

In summary, our study provides the first comprehensive analysis of genetic variants at the ELMO1 locus in a Caucasian population with diabetic nephropathy and type 1 diabetes. Our analysis identified several associations that are independent of those previously identified in other ethnic groups with diabetic nephropathy and type 2 diabetes; however, our examination of this locus in the GoKinD collection further supports its potential role in this disease. Confirmation of the associations identified in our study in additional collections, including ethnically diverse populations with either type 1 or type 2 diabetes, is necessary to better understand the role of these variants, and, perhaps, rare variants yet to be examined may underlie the genetic susceptibility of diabetic nephropathy attributed to this locus.

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No potential conflicts of interest relevant to this article were reported.

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