Variation in SLC19A3 and Protection From Microvascular Damage in Type 1 Diabetes

Diabetes 2016;65:1022–1030 | DOI: 10.2337/db15-1247

The risk of long-term diabetes complications is not fully explained by diabetes duration or long-term glycemic exposure, suggesting the involvement of genetic factors. Because thiamine regulates intracellular glucose metabolism and corrects for multiple damaging effects of high glucose, we hypothesized that variants in specific thiamine transporters are associated with risk of severe retinopathy and/or severe nephropathy because they modify an individual’s ability to achieve sufficiently high intracellular thiamine levels. We tested 134 single nucleotide polymorphisms (SNPs) in two thiamine transporters (SLC19A2/3) and their transcription factors (SP1/2) for an association with severe retinopathy or nephropathy or their combination in the FinnDiane cohort. Subsequently, the results were examined for replication in the DCCT/EDIC and Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) cohorts. We found two SNPs in strong linkage disequilibrium in the SLC19A3 locus associated with a reduced rate of severe retinopathy and the combined phenotype of severe retinopathy and end-stage renal disease. The association for the combined phenotype reached genome-wide significance in a meta-analysis that included the WESDR cohort. These findings suggest that genetic variations in SLC19A3 play an important role in the pathogenesis of severe diabetic retinopathy and nephropathy and may explain why some individuals with type 1 diabetes are less prone than others to develop microvascular complications.

Severe microvascular complications affect more than one-third of people with diabetes (1). Diabetes duration, poor glycemic control, and high blood pressure are the strongest known risk factors for the development of microangiopathy. Although glycemic control may be considered the most important risk factor, a subanalysis of the Diabetes Control and Complications Trial (DCCT) revealed that a three-step change in Early Treatment of Diabetic Retinopathy Study (ETDRS) level occurs in people with sustained optimal levels of HbA1c, whereas some with poor metabolic control did not reach the three-step change outcome during the trial (2). This finding suggests that other factors, possibly genetically determined, contribute to the pathogenesis of microvascular damage. This phenomenon has also been detected in studies showing familial clustering of microvascular complications (3,4). Despite great efforts, only a few genetic loci have been robustly identified with conventional criteria [i.e., discovery $P < 5 \times 10^{-8}$ and independent replication $P < 0.05$].
in the same direction (5–7) for the risk of microvascular complications.

Hyperglycemia may damage blood vessels through multiple biochemical mechanisms, such as overproduction of advanced glycation end products and activation of the polyol hexosamine and diacylglycerol-protein kinase C pathways. In particular, increased production of reactive oxygen species in the Krebs cycle may be a common denominator or unifying mechanism of these pathways (8,9).

Thiamine (vitamin B1) regulates intracellular glucose management through multiple mechanisms (10) and has been shown to correct all of the aforementioned high glucose–induced abnormalities by reducing reactive oxygen species production both in cellular studies (11,12) and in animal models (13). In addition, thiamine and its derivative benfotiamine were shown to reduce the progression of retinopathy and nephropathy in animals with experimental diabetes (14). Hence, impaired thiamine availability may facilitate metabolic damage, and evidence for reduced circulating thiamine levels was described in people with diabetes, possibly secondary to renal loss (15).

Thiamine is carried into the cells by two high-affinity thiamine transporters, hTHTR1 and hTHTR2, and by a low-affinity transporter (16). Two transcription factors, Sp1 and Sp2, are known to affect the expression of SLC19A2/3 encoding hTHTR1/2 (17). Individuals who are susceptible to diabetic retinopathy (DR) and/or diabetic nephropathy (DN) might have an impaired ability to achieve sufficiently high intracellular thiamine levels, which might be particularly relevant in insulin-independent tissues, such as retinal capillary endothelium and pericytes and the neuroretina, because they cannot regulate glucose movement into the cell and are thus exposed to hyperglycemia. We also hypothesized that such a defect may be due to genetic variation in the genes encoding for thiamine transporters and/or their transcription factors.

We examined single nucleotide polymorphisms (SNPs) in the genes encoding for hTHTR1/2 and Sp1/2 in participants with type 1 diabetes in the Finnish Diabetic Nephropathy (FinnDiane) study for association with severe DR compared with no/minimal DR. We also compared participants with various stages of renal damage and the combined phenotype of severe DR and end-stage renal disease (ESRD) versus no/minimal lesions. The results were examined for replication in two independent type 1 diabetes cohorts: the DCCT and its long-term follow-up study Epidemiology of Diabetes Interventions and Complications (EDIC) (18) and the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) (19).

**RESEARCH DESIGN AND METHODS**

**Participants and Phenotype Definitions**

The FinnDiane study is a nationwide multicenter study aimed at detecting genetic and environmental risk factors for diabetic complications in type 1 diabetes. Details on participant recruitment were presented earlier (20). The FinnDiane study includes 3,546 participants with quality-controlled genotype data from a genome-wide association study (GWAS) (5). We included in the analyses participants with type 1 diabetes and minimum duration of 10 years, age at diabetes onset ≤40 years, and insulin treatment initiated within 1 year from diagnosis. The study protocol was approved by the local ethics committees. All participants gave informed consent before participation. The study was performed in accordance with the Declaration of Helsinki.

Analyses for DR included 1,566 cases of severe DR at the baseline visit (defined as ETDRS score ≥53 [severe nonproliferative retinopathy or worse] or any retinal laser treatment) and 218 control subjects with no/mild DR (ETDRS score <35 corresponding to isolated microaneurysms or blot hemorrhages), no laser treatment, and diabetes duration >20 years (Table 1). Correspondingly, participants were divided by stage of DN at the baseline visit, including 415 with ESRD (defined as ongoing dialysis treatment or transplanted kidney), 759 with macroalbuminuria (albumin excretion rate [AER] ≥200 µg/min or ≥300 mg/24 h or albumin-to-creatinine ratio ≥25 mg/mmol for men and ≥35 mg/mmol for women), 442 with microalbuminuria (AER ≥20 <200 µg/min or ≥30 <300 mg/24 h or albumin-to-creatinine ratio 2.5–25 mg/mmol for men and 3.5–35 mg/mmol for women in overnight, 24-h, or spot urine collections), and 1,623 with normal AER (Table 2). Association tests between SNPs and DN (ESRD + macroalbuminuria) versus participants with normal AER, and ESRD versus normal AER were performed. Finally, the combined phenotype comprised participants with both severe DR and ESRD (n = 369) and control subjects with no/minimal DR and no/minimal DN (normal AER or microalbuminuria, n = 190) (Supplementary Table 1). This combined phenotype compares the extreme cases with the most severe irreversible stages of both DN and DR to control subjects whose status in terms of both DN and DR shows only the very first signs of the complications, which could still regress to normal states. Furthermore, major progression is less likely to occur in subjects with a long diabetes duration.

The significant associations were replicated in independent cohorts from the DCCT/EDIC (18) and WESDR (19) studies. Participant recruitment and cohort summaries for both were presented earlier (18,19).

Both replication studies used the latest available participant data to achieve the long duration of diabetes required for the present study. The WESDR cohort had 311 patient cases and 157 control subjects for the DR analyses. Of these, 82 cases and 112 controls were suitable for the analysis of the combined phenotype. Only participants with type 1 diabetes were included in the analyses. In the DCCT/EDIC cohort, 209 cases and 228 controls were included for the analysis of DR. In the DCCT/EDIC, we performed the analysis in three subgroups defined by cohort and treatment group assignment: primary prevention cohort (53 cases vs. 58 controls, models adjusted for DCCT randomized treatment); secondary cohort–conventional treatment
Variation in SLC19A3 and Microvascular Damage

Case-control association testing was performed with Statistical Calculations regions contained 134 SNPs: 40 in the FinnDiane data were previously described (5). The re-
genotyping, quality control, and genotype imputation of imputed with HapMap II reference panel. The genome-wide the existing FinnDiane genome-wide genotyping data im-
10 kilobases up- and downstream, were extracted from puted from the DCCT/EDIC and WESDR, whereas rs12694743 SNPs selected for replication, rs6713116 was directly geno-
typed in both DCCT/EDIC and WESDR, whereas rs12694743 typed in both DCCT/EDIC and WESDR, whereas rs12694743 was imputed with good quality (WESDR: 0.96, DCCT/EDIC: imputed with good quality (WESDR: 0.96, DCCT/EDIC: 0.97) using HapMap II r22 as the reference.

## Genotyping
SNPs within the four candidate gene regions, including 10 kilobases up- and downstream, were extracted from the existing FinnDiane genome-wide genotyping data im-
puted with HapMap II reference panel. The genome-wide genotyping, quality control, and genotype imputation of the FinnDiane data were previously described (5). The regions contained 134 SNPs: 40 in SLC19A2, 46 in SLC19A3, 23 in SP1, and 25 in SP2. Details on genotyping, quality control, and genotype imputation measures in DCC/EDIC and WESDR have been described elsewhere (7). Of the two SNPs selected for replication, rs6713116 was directly geno-
typed in both DCCT/EDIC and WESDR, whereas rs12694743 was imputed with good quality (WESDR: 0.96, DCCT/EDIC: 0.97) using HapMap II r22 as the reference.

## Statistical Calculations
Case-control association testing was performed with an additive logistic regression model using PLINK v1.07 (21), with genotypes defined as continuous allelic dosages. The analyses were primarily adjusted for age, diabetes du-
ration, sex, and the first 10 genetic principal components (PCs) computed with EIGENSTRAT software (EIGENSOFT v3.0) (22).

Significant associations were further reanalyzed by corresponding logistic regression models adjusted for risk factors, such as HbA1c, BMI, plasma lipids, and blood pressure, measured at the baseline visit. Models were expanded step by step, adding significant covariates from each class of similar biomarkers if they improved the model fit by Akaike information criterion. Covariates with skewed distributions were log-transformed.

In the DCCT/EDIC cohort, in addition to age, sex, and duration of diabetes at the last follow-up visit, only the first three PCs were included as covariates in the models. Additional covariates in the extended model were BMI (latest follow-up visit) and mean HbA1c in both replication cohorts. Both replication cohorts used mean HbA1c during the entire follow-up period. The results were merged with the FinnDiane data in a fixed-effects meta-analysis by using METAL software (23), with the inverse variance–based method using effect size estimates and their SEs. The heterogeneity of the cohorts was evaluated by using the Cochran Q test and the corresponding I² statistic implemented in the METAL software. We estimated the replication power with the Genetic Power Calculator (24) based on estimates of relative risk (RR) and disease prevalence evaluated from the Finn-
diane and detected allele frequencies from the DCCT/EDIC and WESDR. Odds ratios (ORs) resulting from the ba-
sic models were equated to RR by using Eq. 1:

\[
RR = OR/[(1 - P_0) + (P_0 \times OR)]
\]  

where \( P_0 \) is the incidence in the nonexposed group (high-risk genotype). To account for the winner’s curse, we used 60% decreased effect size to achieve a bias-reduced estimate for power (25,26).

## Adjustment for Multiple Testing
To account for multiple statistical tests, the threshold for statistical significance was adjusted with Bonferroni correction. This correction included the initial tests performed for 134 SNPs by using the basic model and four different phenotypes (4 × 134 = 536 tests). In addition, it included the tests from the four extended models used to analyze the two SNPs found associated with two of the phenotypes (4 × 2 × 2 = 16 additional tests). Thus, the significance threshold was set at \( P < 0.05 / 552 \approx 9.06 \times 10^{-5} \).

### Table 1—Clinical characteristics of the participants included in the association analysis of the DR phenotype in the FinnDiane cohort

| Characteristic          | Severe DR | No/mild DR | \( P \) value* |
|------------------------|-----------|------------|----------------|
| Sex (n [male/female])  | 1,566 (886/680) | 218 (91/127) | < 0.001        |
| Mean ± SD Missing (r) |           |            |                |
| Age (years)            | 44.4 ± 10 | 41.0 ± 10  | < 0.001        |
| Duration (years)       | 31.6 ± 8.8| 28.3 ± 6.9 | < 0.001        |
| HbA1c (%)              | 8.7 ± 3.6 | 8.2 ± 3.3  | < 0.001        |
| HbA1c (mmol/mol)       | 71.1 ± 16 | 65.6 ± 13  | < 0.001        |
| BMI                    | 25.4 ± 3.9| 25.3 ± 3.1 | 0.78           |
| TG (mmol/L)            | 1.52 ± 1.1| 1.1 ± 0.6  | < 0.001        |
| SBP (mmHg)             | 144 ± 21  | 133 ± 19   | < 0.001        |
| DBP (mmHg)             | 82 ± 11   | 79 ± 9     | < 0.001        |

DBP, diastolic blood pressure; SBP, systolic blood pressure; TG, triglyceride. *P values for difference between groups. \( P \) value for sex evaluated by using Pearson \( \chi^2 \) test (1 degree of freedom). \( P \) values for continuous traits evaluated by using Welch two-sample \( t \) test.
SNP Association With Covariates
We tested whether the top SNPs were associated with other clinical covariates by using univariate linear regression. Testing was performed both in the 462 FinnDiane participants included in the analysis of the combined phenotype (with complete data) and in all genotyped FinnDiane participants (number varying due to missing data and reported separately for each covariate) (Supplementary Table 3).

RESULTS
Two SNPs in the intronic region of SLC19A3 in strong linkage disequilibrium (LD) with each other (FinnDiane \( r^2 = 0.93 \)) showed a significant association with DR: rs12694743 \((P = 3.81 \times 10^{-6}, \text{OR} 0.51 \) [95% CI 0.38–0.68]) and rs6713116 \((P = 3.15 \times 10^{-6}, \text{OR} 0.44 \) [0.28–0.60]) (Table 3). The minor alleles of both SNPs were associated with lower risk for severe DR.

No significant association \((P < 9.06 \times 10^{-5})\) was detected with the DN or ESRD phenotypes in any of the studied genes (data not shown). Nevertheless the same two SNPs showed nominal significance when comparing participants with normal AER and ESRD \((\text{rs12694743: } P = 8.9 \times 10^{-4}, \text{OR} 0.62 \) [95% CI 0.47–0.82]; \(\text{rs6713116: } P = 1.3 \times 10^{-3}, \text{OR} 0.56 \) [0.38–0.80]). Minor alleles were associated with a lower risk of complications. The same two SNPs were also strongly associated with the combined phenotype of severe DR and ESRD \((\text{rs12694743: } P = 7.51 \times 10^{-8}, \text{OR} 0.31 \) [0.20–0.47]; \(\text{rs6713116: } P = 7.49 \times 10^{-7}, \text{OR} 0.26 \) [0.15–0.44]) (Table 4).

The \(P\) values and ORs were virtually unchanged when adjusted for additional covariates in the DR phenotype; however, adding covariates to the model of the combined phenotype gradually increased the protective effect of the SNPs (i.e., the OR decreased) (Supplementary Table 2). No significant association was found between the top two SNPs and any clinical covariates in the FinnDiane participants (Supplementary Table 3) in the aforementioned subsample (Supplementary Table 4) or in the publicly available studies of large consortia [HbA1c (27), BMI (28), serum triglycerides (29), and systolic/diastolic blood pressure (30)]. The other genetic regions analyzed (SLC19A2, SP1/2) showed no significant associations with any of the phenotypes.

Replication
The association of these two SNPs with DR phenotype was not replicated in the WESDR and DCCT/EDIC cohorts (Table 3). Adjusting the models for mean HbA1c and BMI did not alter the results (data not shown). The results of the combined meta-analysis of the replication cohorts and the FinnDiane were not significant (Table 3, Fig. 1), yet there was significant evidence for heterogeneity of the effect across the cohorts \((\text{rs12694743: } I^2 = 64, P = 0.02; \text{rs6713116: } I^2 = 71, P = 0.008 \text{ by Cochran Q test}).\)

Because of the limited number of ESRD cases in the DCCT/EDIC, only the WESDR cohort was tested for replication of the association for the combined phenotype. Both SNPs were significantly associated with the combined phenotype \((P < 0.05)\) (Table 4). Adjusting the models for BMI and mean HbA1c in WESDR made the association nonsignificant \((P > 0.05)\), arguing for some level of mediation. When the replication results were combined with the FinnDiane data in meta-analysis, rs12694743 reached genome-wide significance \((P < 5 \times 10^{-8})\) both before \((P = 7.14 \times 10^{-5})\) and after \((P = 2.30 \times 10^{-8})\) adjustment for BMI and HbA1c (Table 4). The SNPs had similar effect size estimates in both WESDR and FinnDiane (Table 4, Fig. 1), and there was no evidence for heterogeneity (Cochran Q test).

DISCUSSION
We investigated the hypothesis that variations in the genes encoding the high-affinity membrane thiamine transporters hTHTR1/2 or their transcription factors Sp1/2 may affect susceptibility to developing microvascular complications of diabetes. Our hypothesized mechanism is that this is due

---

Table 2—Clinical characteristics of participants included in the association analysis of the DN phenotype in the FinnDiane cohort divided by DN status

| Characteristic | Normal AER | Microalbuminuria | Macrolalbuminuria | ESRD | \(P\) value* |
|---------------|------------|------------------|-------------------|------|-------------|
| Sex (n [male/female]) | 1,623 (679/944) | 442 (253/189) | 759 (453/306) | 415 (248/167) | < 0.001 |
| Age (years) | 40.9 ± 12 | 40.4 ± 12 | 43.1 ± 10 | 45.7 ± 8.8 | < 0.001 |
| Duration (years) | 25.7 ± 10 | 27.9 ± 10 | 29.9 ± 8.7 | 33.0 ± 8.5 | < 0.001 |
| HbA1c (%) | 8.2 ± 3.4 | 8.2 ± 3.6 | 9.0 ± 3.7 | 7.0 ± 3.8 | < 0.001 |
| BMI | 25.0 ± 3.4 | 25.9 ± 3.6 | 26.0 ± 4.0 | 24.1 ± 3.8 | 0.89 |
| TG (mmol/L) | 1.06 ± 0.7 | 1.33 ± 0.9 | 1.71 ± 1.1 | 1.69 ± 1.0 | 0.001 |
| SBP (mmHg) | 131 ± 17 | 138 ± 17 | 145 ± 20 | 152 ± 24 | 0.001 |
| DBP (mmHg) | 78 ± 9 | 81 ± 10 | 83 ± 10 | 84 ± 12 | 0.001 |

DBP, diastolic blood pressure; SBP, systolic blood pressure; TG, triglyceride. \(P\) values for difference between groups.
Table 3—Significant results of association analyses with the DR phenotype in the FinnDiane cohort, results of replication cohorts and meta-analyses

| chr | SNP | bp | Minor | Major | Cohort | Imputation quality | Participants (n) | MAF | OR* (95% CI) | P value |
|-----|-----|----|-------|-------|--------|-------------------|-----------------|-----|--------------|---------|
|     |     |    |       |       |        |                   |                 |     |              |         |
| 2   | rs12694743 | 228,266,664 | G | A | FinnDiane | 0.93 | 1,566 | 218 | 1,784 | 0.14 | 0.22 | 0.15 | 0.51 (0.38–0.68) | 3.81 × 10⁻⁶ |
|     |     |    |       |       |        |                   |                 |     |              |         |
|     |     |    |       |       |        |                   |                 |     |              |         |
| 2   | rs6713116 | 228,275,342 | T | C | FinnDiane | 0.81 | 1,566 | 218 | 1,784 | 0.15 | 0.21 | 0.16 | 0.41 (0.28–0.60) | 3.15 × 10⁻⁶ |

bp, base pair; chr, chromosome; DCCT¹, DCCT primary cohort; DCCT², secondary cohort–conventional treatment; DCCT³, secondary cohort–intensive treatment; MAF, minor allele frequency. *OR of logistic regression for each copy of minor allele.

We observed a strong association of rs12694743 and rs6713116 in SLC19A3 with severe DR and ESRD. Furthermore, the combined phenotype of severe DR and ESRD was not tested because of an insufficient number of ESRD cases. Both strength and direction of the associations were similar in both cohorts, and no evidence for heterogeneity of the SNP effects in the FinnDiane cohort was found. The additional model adjustment for type of severe DR and ESRD (DR, ESRD = 7.5 × 10⁻⁶) did not change the results significantly.
A limitation of this study is that it only focused on the known high-affinity thiamine transporter genes, whereas additional variants in related functional pathways may exist in different populations. We cannot comment on the gene expression of these variants in this study. However, we could not detect all the expressed variants. Therefore, these SNPs possibly represent binding in the region and the expression of regulatory protein possibly even in another gene not genotyped or imputed. Another possibility is that these variants affect regulatory protein binding from marrow stem cells, pancreatic 

# Table 4

| chr SNP | Minor Major | P value | Allele Patient Quality | Case Control Total | OR* (95% CI) |
|---------|-------------|---------|------------------------|--------------------|--------------|
| 2 16571316 | 228.37 | 0.46 | 0.95 | Case Control | 0.96 (0.93–0.99) |
| 2 162947134 | 228.38 | 0.66 | 0.96 | Case Control | 0.96 (0.93–0.99) |

Participants with ESRD also had severe retinopathy, the more important aspect of the combined phenotype is that the control subjects seem to have something protecting them from complications, 

Thiamine acts as a coenzyme for three key enzymes in the Krebs cycle (10). A limitation of this study is that it only focused on the known high-affinity thiamine transporter genes, whereas additional variants in related functional pathways may exist in different populations. We cannot comment on the gene expression of these variants in this study. However, we could not detect all the expressed variants. Therefore, these SNPs possibly represent binding in the region and the expression of regulatory protein possibly even in another gene not genotyped or imputed. Another possibility is that these variants affect regulatory protein binding from marrow stem cells, pancreatic 

**Table 4**

| chr SNP | Minor Major | P value | Allele Patient Quality | Case Control Total | OR* (95% CI) |
|---------|-------------|---------|------------------------|--------------------|--------------|
| 2 16571316 | 228.37 | 0.46 | 0.95 | Case Control | 0.96 (0.93–0.99) |
| 2 162947134 | 228.38 | 0.66 | 0.96 | Case Control | 0.96 (0.93–0.99) |

Participants with ESRD also had severe retinopathy, the more important aspect of the combined phenotype is that the control subjects seem to have something protecting them from complications, showing only no/minimal DR/DN after 20 years of diabetes is uncommon. The associated SNPs are located in the intronic regions of SLC19A3 (16571316 in the Danish and 162947134 in the Finnish cohort). However, we could not find the any functional annotation or regulatory elements underlying the associated SNPs. Therefore, these SNPs possibly represent binding in the region and the expression of regulatory protein possibly even in another gene not genotyped or imputed.
independent cohorts with similar phenotypic data, and the lead SNP reached genome-wide significant association with the combined phenotype of severe DR and ESRD in meta-analysis. However, the number of subjects who fit our strict phenotype definitions was small in the WESDR cohort, and the evidence was only modest if inspected independently, making the small replication a major limitation of this study. Furthermore, the strict phenotype inclusion criteria for the combined phenotype did not permit further replication in other type 1 diabetes cohorts mainly because of a lack of ETDRS-based DR classifications.

Although the results indicate a plausible explanation for why hyperglycemia in some patients with type 1 diabetes causes harm while others are protected from its deleterious effects, clinical trials with benfotiamine in microvascular complications have notably failed to show a protective effect. However, those trials have been short term and have included small numbers of subjects (37,38), with some studies showing contradictory results (39). Another important aspect is that these trials have not targeted the same phenotype as shown to be associated with the genetic variants in the present analysis. Clinical trials on thiamine have also shown successful results for treating nephropathy as reviewed previously (40). Therefore, we cannot yet draw a final conclusion on a potential clinical benefit of targeted treatment with vitamin B1 agents.

Because the SNPs were not associated with other known risk factors affecting DR/DN and the associations with DR/DN phenotypes remain even after adjustment for covariates, the SNPs appear to represent a novel independent risk factor for both complications. However, these results should be studied further in additional cohorts and/or by other methods to confirm them and to gain additional insight on the associated SNPs’ function. Finally, the results and observations fit well with the original working hypothesis, providing a novel interpretation for the pathogenesis of DR/DN. In particular, these results help to explain why some individuals are less prone than others to develop the microvascular complications of diabetes and might lead to the early identification of those at risk.

Acknowledgments. The authors thank the staff and participants of the FinnDiane, DCCT/EDIC, and WESDR.

Funding. A.D.P. holds a Canada Research Chair in the Genetics of Complex Diseases. FinnDiane was supported by grants from the Folkhälsof Research Foundation, Wilhelm and Else Stockmann Foundation, Liv och Hälsoa Foundation, Helsinki University Central Hospital Research Funds (EVO), Finnish Cultural Foundation, Signe and Aale Gyllenberg Foundation, Novo Nordisk Foundation, Academy of Finland, Tekes, and Finnish Medical Society (Finska Läkaresällskapet). The DCCT/EDIC Research Group is sponsored through research contracts from the Division of Diabetes, Endocrinology, and Metabolic Diseases of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institutes of Health. Clinical data and DNA from the DCCT/EDIC study is available through the NIDDK repository at www.niddkrepository.org/niddk/home.do. A.D.P. is the guarantor for DCCT/EDIC. The DCCT/EDIC has been supported by U01 Cooperative Agreement grants (1982–1993, 2011–2016) and contracts (1982–2011) with the Division of Diabetes, Endocrinology, and Metabolic Diseases of the NIDDK (current grant numbers U01-DK-094176 and U01-DK-094157) and through support by the National Eye Institute, National Institute of Neurological Disorders and Stroke, General Clinical Research Centers Program (1993–2007), and Clinical Translational Science Center Program (2006–present), Bethesda, MD (Clinical trial reg. nos. NCT00360815 and NCT00360893, clinicaltrials.gov). Additional support for this DCCT/EDIC collaborative study was provided by grants from NIDDK contract N01-DK-62204; NIDDK grants R01-DK-077510, R01-DK-077489, and P60-DK-20595; and support from Genome Canada through the Ontario Genomics Institute. The WESDR was supported by grant R01-EY-016379 from the National Eye Institute, National Institutes of Health.

| Severe DR: $\text{rs}12694743$ | Severe DR + ESRD: $\text{rs}12694743$ |
|---------------------------|----------------------------------|
| **Cohort**                | **OR [CI 95%]**                  | **Cohort**                | **OR [CI 95%]**                  |
| FINNDB                    | 0.51 [0.38, 0.68]                | FINNDB Basic              | 0.31 [0.20, 0.47]                |
| WESDR                     | 1.14 [0.73, 1.80]                | WESDR Basic               | 0.34 [0.13, 0.91]                |
| DCCT1                     | 1.26 [0.53, 3.14]                | WESDR Adjusted            | 0.29 [0.09, 1.10]                |
| DCCT2                     | 0.81 [0.42, 1.66]                | Meta: Basic               | 0.32 [0.22, 0.47]                |
| DCCT3                     | 0.64 [0.26, 1.57]                | Meta: Adjusted            | 0.28 [0.16, 0.44]                |

**Figure 1**—Forest plots of replication results and meta-analyses for lead SNPs in the analysis of DR and the combined phenotype. The point size of the diamonds and the squares are proportional to the precision of the estimates of OR. Results for $\text{rs}6713116$ behaved similarly. DCCT1, DCCT primary cohort; DCCT2, DCCT secondary cohort—conventional treatment; DCCT3, DCCT secondary cohort—intensive treatment.
The content is solely the responsibility of the authors and does not necessarily reflect the official views of the NIDDK.

**Duality of Interest.** M.P. has received honoraria as an advisory board member from Abbott, Novartis, Roche Diagnostics, and Sanofi. K.H. has received lecture honoraria from Santen and Allergan and has been an advisory board member of Allergan. P.-H.G. has received lecture honoraria from AbbVie, Boehringer Ingelheim, Cellicx, Eli Lilly, Genzyme, Novartis, NordiKis, MSD, and Medscape and research grants from Eli Lilly and Roche. P.-H.G. also is an advisory board member of Boehringer Ingelheim, Eli Lilly, and Novartis. Industry contributors playing no role in the DCCT/EDIC study but having provided free or discounted supplies or equipment to support participants’ adherence to the study were Abbott Diabetes Care (Alameda, CA), Animlas (Westchester, PA), Bayer Diabetes Care (North America Headquarters, Tarrytown, NY), Becton Dickinson (Franklin Lakes, NJ), CanAm (Atlanta, GA), Eli Lilly (Indianapolis, IN), LifeScan (Milpitas, CA), Medtronic Diabetes (Minneapolis, MI), Nova Diabetes Care (Billenica, MA), Omron (Shelton, CT), OmniPod Insulin Management System (Bedford, MA), Roche Diabetes Care (Indianapolis, IN), and Sanofi (Bridgewater, NJ). No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** M.P. contributed to the study design, drafting of the manuscript, and review and editing of the manuscript. I.T. analyzed data from FinnDiane, combined the results in the meta-analysis, and contributed to the drafting and review and editing of the manuscript. N.S. prepared the genotype data in FinnDiane and contributed to the review and editing of the manuscript. S.M.H. analyzed data from DCCT/EDIC and WESDR and contributed to the review and editing of the manuscript. C.F., K.H., and V.H. collected data in FinnDiane and contributed to the study design and review and editing of the manuscript. B.E.K., R.K., and A.D.P. contributed to the review and editing of the manuscript. L.B. and P.-H.G. contributed to the study design and review and editing of the manuscript. C.F., K.H., and V.H. collected data in FinnDiane and contributed to the review and editing of the manuscript. I.T. analyzed data from DCCT/EDIC and WESDR and contributed to the review and editing of the manuscript. S.M.H. analyzed data from DCCT/EDIC and WESDR and contributed to the review and editing of the manuscript. C.F., K.H., and V.H. collected data in FinnDiane and contributed to the review and editing of the manuscript. B.E.K., R.K., and A.D.P. contributed to the review and editing of the manuscript. L.B. and P.-H.G. contributed to the study design and review and editing of the manuscript. P.-H.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented in abstract form at the 50th European Association for the Study of Diabetes Annual Meeting, Vienna, Austria, 15–19 September 2014.

**References**

1. Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med 1993;328:1676–1685
2. The Diabetes Control and Complications Trial Research Group. The absence of a glycemic threshold for the development of long-term complications: the perspective of the Diabetes Control and Complications Trial. Diabetes 1996;45:1289–1298
3. Hietala K, Forsblom C, Summanen P, Groop PH. FinnDiane Study Group. Heritability of proliferative diabetic retinopathy. Diabetes 2008;57:2176–2180
4. Harjutsalo V, Katoh S, Sarti C, Tajima N, Tuomilehto J. Population-based assessment of familial clustering of diabetic nephropathy in type 1 diabetes. Diabetes 2004;53:2449–2454
5. Sandholm N, Salem RM, McKnight AJ, et al.; DCCT/EDIC Research Group. New susceptibility loci associated with kidney disease in type 1 diabetes. PLoS Genet 2012;8:e1002921
6. Sandholm N, McKnight AJ, Salem RM, et al.; FIND Consortium; FinnDiane Study Group and the GENIE Consortium. Chromosome 2q31.1 associates with ESRD in women with type 1 diabetes. J Am Soc Nephrol 2013;24:1537–1543
7. Hosseini SM, Boright AP, Sun L, et al.; DCCT/EDIC Research Group. The association of previously reported polymorphisms for microvascular complications in a meta-analysis of diabetic retinopathy. Hum Genet 2015;134:247–257
8. Nishikawa T, Edelstein D, Brownlee M. The linking missing link: a single unifying mechanism for diabetic complications. Kidney Int Suppl 2000;77:S26–S30
9. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625
10. Singleton CK, Martin PR. Molecular mechanisms of thiamine utilization. Curr Mol Med 2001;1:197–207
11. La Selva M, Beltramo E, Pagnozzi F, et al. Thiamine corrects delayed repilation and decreases production of lactate and advanced glycation end-products in bovine retinal and human umbilical vein endothelial cells cultured under high glucose conditions. Diabetologia 1996;39:1263–1268
12. Berrone E, Beltramo E, Solimine C, Ape AU, Porta M. Regulation of intracellular glucose and polyol pathway by thiamine and benfotiamine in vascular cells cultured in high glucose. J Biol Chem 2006;281:9307–9313
13. Hammes HP, Du X, Edelstein D, et al. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic nephropathy. Nat Med 2003;9:294–299
14. Babaie-Jadidi R, Karachalios N, Ahmed N, Battah S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. Diabetes 2003;52:2110–2120
15. Thornalley PJ, Babaie-Jadidi R, Al Ali H, et al. High prevalence of low plasma thiamine concentration in diabetes linked to a marker of vascular disease. Diabetologia 2007;50:2164–2170
16. Jurgenson CT, Begley TP, Ealick SE. The structural and biochemical foundations of thiamin biosynthesis. Annu Rev Biochem 2009;78:569–603
17. Nabokina SM, Said HM. Characterization of the 5′-regulatory region of the human thiamin transporter SLC19A3: in vitro and in vivo studies. Am J Physiol Gastrointest Liver Physiol 2004;287:G622–G629
18. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. JAMA 2002;287:2563–2569
19. Klein R, Klein BE, Moss SE. Epidemiology of proliferative diabetic retinopathy. Diabetes Care 1992;15:1875–1891
20. Thorn LM, Forsblom C, Fagerudd J, et al.; FinnDiane Study Group. Metabolic syndrome in type 1 diabetes: association with diabetic nephropathy and glycemic control (the FinnDiane study). Diabetes Care 2005;28:2019–2024
21. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575
22. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38:904–909
23. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 2010;26:2190–2191
24. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–150
25. Ioannidis JP, Thomas G, Daly MJ. Validating, augmenting and refining genome-wide association signals. Nat Rev Genet 2008;9:306–315
26. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 2010;26:2190–2191
27. Verrelli C, Mauro M, Di Marzo E, et al. Common variants at 10 genomic loci contribute to susceptibility to myocardial infarction. Nat Genet 2007;39:920–929
28. Speliotes EK, Willer CJ, Berndt SI, et al.; MAGIC; Procardis Consortium. Genome-wide association analysis of serum triglyceride levels identifies a novel susceptibility locus at 9p21. Am J Hum Genet 2010;87:816–825
29. Speliotes EK, Willer CJ, Berndt SI, et al.; MAGIC; Procardis Consortium. Genome-wide association analysis of serum triglyceride levels identifies a novel susceptibility locus at 9p21. Am J Hum Genet 2010;87:816–825
30. Cannon-Albright LA, McGuckin ME, Rapp RH, et al. Genetic susceptibility to several autoimmune diseases. Am J Hum Genet 1995;57:936–945
31. Schmid H, Boucherot A, Yasuda Y, et al.; European Renal cDNA Bank (ERCB) Consortium. Modular activation of nuclear factor-kappaB transcriptional programs in human diabetic nephropathy. Diabetes 2006;55:2993–3003
32. Eudy JD, Spiegelstein O, Barber RC, Wlodarczyk BJ, Talbot J, Finnell RH. Identification and characterization of the human and mouse SLC19A3 gene: a novel member of the reduced folate family of micronutrient transporter genes. Mol Genet Metab 2000;71:581–590
33. Rajgopal A, Edmondson A, Goldman ID, Zhao R. SLC19A3 encodes a second thiamine transporter ThTr2. Biochim Biophys Acta 2001;1537:175–178
34. Rindi G, Laforenza U. Thiamine intestinal transport and related issues: recent aspects. Proc Soc Exp Biol Med 2000;224:246–255
35. Ozand PT, Gascon GG, Al Essa M, et al. Biotin-responsive basal ganglia disease: a novel entity. Brain 1998;121:1267–1279
36. Kono S, Miyajima H, Yoshida K, Togawa A, Shirakawa K, Suzuki H. Mutations in a thiamine-transporter gene and Wernicke’s-like encephalopathy. N Engl J Med 2009;360:1792–1794
37. Alkhalaf A, Kleefstra N, Groenier KH, et al. Effect of benfotiamine on advanced glycation endproducts and markers of endothelial dysfunction and inflammation in diabetic nephropathy. PLoS One 2012;7:e40427
38. Alkhalaf A, Klooster A, van Oeveren W, et al. A double-blind, randomized, placebo-controlled clinical trial on benfotiamine treatment in patients with diabetic nephropathy. Diabetes Care 2010;33:1598–1601
39. Du X, Edelstein D, Brownlee M. Oral benfotiamine plus α-lipoic acid normalises complication-causing pathways in type 1 diabetes. Diabetologia 2008;51:1930–1932
40. Rabbani N, Thornalley PJ. Emerging role of thiamine therapy for prevention and treatment of early-stage diabetic nephropathy. Diabetes Obes Metab 2011;13:577–583