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

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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 HbA_{1c}, 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 $P < 0.05$

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*A complete list of participants in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group is provided in the Supplementary Data online and can be found in N Engl J Med 2011;365:2366–2376. A complete list of physicians and nurses at each center participating in the collection of participants in the Finnish Diabetic Nephropathy (FinnDiane) study is provided in the Supplementary Data online.

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 ethnics 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

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 (*n*)	Mean ± SD	Missing (*n*)	
Age (years)	44.4 ± 10	—	41.0 ± 10	—	< 0.001
Duration (years)	31.6 ± 8.8	—	28.3 ± 6.9	—	< 0.001
HbA_{1c} (%) HbA_{1c} (mmol/mol)	8.7 ± 3.6 71.1 ± 16	42	8.2 ± 3.3 65.6 ± 13	—	< 0.001
BMI	25.4 ± 3.9	181	25.3 ± 3.1	—	0.78
TG (mmol/L)	1.52 ± 1.1	45	1.1 ± 0.6	—	< 0.001
SBP (mmHg)	144 ± 21	260	133 ± 19	2	< 0.001
DBP (mmHg)	82 ± 11	263	79 ± 9	2	< 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 χ^2 test (1 degree of freedom). *P* values for continuous traits evaluated by using Welch two-sample *t* test.

(114 cases vs. 51 controls); and secondary cohort–intensive treatment (42 cases vs. 119 controls). Because the DCCT/EDIC cohort had only a few participants with ESRD, it could not be used to replicate the results of the combined phenotype.

Genotyping

SNPs within the four candidate gene regions, including 10 kilobases up- and downstream, were extracted from the existing FinnDiane genome-wide genotyping data imputed 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 DCCT/EDIC and WESDR have been described elsewhere (7). Of the two SNPs selected for replication, rs6713116 was directly genotyped 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 duration, sex, and the first 10 genetic principal components (PCs) computed with EIGENSTRAT software (EIGENSOFT v.3.0) (22).

Significant associations were further reanalyzed by corresponding logistic regression models adjusted for risk factors, such as HbA_{1c}, 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 HbA_{1c} in both replication cohorts. Both replication cohorts used mean HbA_{1c} 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 I^2 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 FinnDiane and detected allele frequencies from the DCCT/EDIC and WESDR. Odds ratios (ORs) resulting from the basic models were equated to RR by using Eq. 1:

$$RR = OR/[(1 - P_0) + (P_0 \times OR)] \quad \text{(Eq. 1)}$$

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 ($4 \times 134 = 536$ 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 ($4 \times 2 \times 2 = 16$ additional tests). Thus, the significance threshold was set at $P < 0.05 / 552 \cong 9.06 \times 10^{-5}$.

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		Macroalbuminuria		ESRD		*P* value*
Sex (*n* [male/female])	1,623 (679/944)		442 (253/189)		759 (453/306)		415 (248/167)		< 0.001
	Mean ± SD	Missing	Mean ± SD	Missing	Mean ± SD	Missing	Mean ± SD	Missing	
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
HbA_{1c} (%) HbA_{1c} (mmol/mol)	8.2 ± 3.4 65.9 ± 14	62	8.2 ± 3.6 65.9 ± 16	2	9.0 ± 3.7 75.0 ± 17	40	8.6 ± 3.8 70.9 ± 18	26	< 0.001
BMI	25.0 ± 3.4	128	25.9 ± 3.6	30	26.0 ± 4.0	145	24.1 ± 3.8	90	0.89
TG (mmol/L)	1.06 ± 0.7	35	1.33 ± 0.9	7	1.71 ± 1.1	55	1.69 ± 1.0	35	< 0.001
SBP (mmHg)	131 ± 17	193	138 ± 17	42	145 ± 20	206	152 ± 24	106	< 0.001
DBP (mmHg)	78 ± 9	194	81 ± 10	42	83 ± 10	207	84 ± 12	108	< 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 χ^2 test (3 degrees of freedom). *P* values for continuous traits evaluated by using one-way ANOVA.

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 ($P = 3.81 \times 10^{-6}$, OR 0.51 [95% CI 0.38–0.68]) and rs6713116 ($P = 3.15 \times 10^{-6}$, 0.41 [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 (rs12694743: $P = 8.9 \times 10^{-4}$, OR 0.62 [95% CI 0.47–0.82]; rs6713116: $P = 1.3 \times 10^{-3}$, 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 (rs12694743: $P = 7.51 \times 10^{-8}$, 0.31 [0.20–0.47]; rs6713116: $P = 7.49 \times 10^{-7}$, 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 [HbA_{1c} (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 HbA_{1c} 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 (rs12694743: I^2 = 64, *P* = 0.02; rs6713116: I^2 = 71, *P* = 0.008 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 HbA_{1c} 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 ($P = 7.14 \times 10^{-9}$) and after ($P = 2.30 \times 10^{-8}$) adjustment for BMI and HbA_{1c} (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 3—Significant results of association analyses with the DR phenotype in the FinnDiane cohort, results of replication cohorts and meta-analyses

chr	SNP	bp	Allele		Cohort	Imputation quality	Participants (*n*)			MAF			OR* (95% CI)	*P* value
			Minor	Major			Case	Control	Total	Case	Control	All		
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}
					WESDR	0.96	311	157	468	0.12	0.12	0.12	1.14 (0.73–1.80)	0.57
					DCCT[1]	0.97	53	58	111	0.18	0.17	0.18	1.29 (0.53–3.14)	0.58
					DCCT[2]	0.97	114	51	165	0.11	0.15	0.12	0.81 (0.42–1.56)	0.52
					DCCT[3]	0.97	42	119	161	0.09	0.13	0.12	0.64 (0.26–1.57)	0.33
					Meta-analysis: replication cohorts	—	520	385	905	0.12	0.13	0.13	0.99 (0.72–1.37)	0.96
					Meta-analysis: all cohorts combined	—	2,086	603	2,689	0.14	0.16	0.14	0.68 (0.55–0.85)	4.76×10^{-4}
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}
					WESDR	1	311	157	468	0.13	0.14	0.14	1.01 (0.67–1.54)	0.95
					DCCT[1]	1	53	58	111	0.22	0.21	0.21	1.33 (0.57–3.10)	0.51
					DCCT[2]	1	114	51	165	0.14	0.16	0.15	0.93 (0.5–1.74)	0.82
					DCCT[3]	1	42	118	160	0.12	0.16	0.15	0.73 (0.34–1.58)	0.42
					Meta-analysis: replication cohorts	—	520	384	904	0.14	0.16	0.15	0.98 (0.73–1.32)	0.89
					Meta-analysis: all cohorts combined	—	2,086	602	2,688	0.15	0.18	0.16	0.70 (0.56–0.88)	2.74×10^{-3}

bp, base pair; chr, chromosome; DCCT[1], DCCT primary cohort; DCCT[2], secondary cohort–conventional treatment; DCCT[3], secondary cohort–intensive treatment; MAF, minor allele frequency. *OR of logistic regression for each copy of minor allele.

to an enhanced capability of handling high intracellular glucose through thiamine-modulated pathways in insulin-independent tissues.

We observed a strong association of rs12694743 and rs6713116 in *SLC19A3* with severe DR ($P_{lead} = 3.8 \times 10^{-6}$) and an even stronger association with the merged phenotype of severe DR and ESRD ($P_{lead} = 7.5 \times 10^{-8}$) in the FinnDiane cohort. The severe DR results could not be replicated. Still, the combined phenotype of severe DR and ESRD showed associations in the WESDR cohort (DCCT/EDIC was not tested because of an insufficient number of ESRD cases), and rs12694743 reached genome-wide significance in the meta-analysis ($P = 7.1 \times 10^{-9}$). Both strength and direction of association were similar, further model adjustments behaved similarly in both cohorts, and no evidence for heterogeneity of the SNP effects for the combined phenotype was found. Moreover, the additional model adjustments in the FinnDiane cohort suggested that the protective effect is independent of cross-sectionally measured known risk factors of DN and DR. Even though the adjustment resulted in exclusion of some individuals due to missing data, there should be no selection bias affecting the results because the allele frequencies in the participant subsamples with nonmissing data did not change (Supplementary Table 2). No associations with other studied genetic regions were found.

Although the association with severe DR could not be replicated, we estimated that after adjustment for the winner's curse, there was only 31% power to detect corresponding lead associations ($P < 0.05$) in the combined set of replication cohorts, suggesting that lack of replication may be due to insufficient statistical power. Thus, even when all the replication cohorts are combined, the numbers are possibly too small to detect the marginal effect of DR or DN alone (i.e., the association with just one of the phenotypes). As the analysis in the FinnDiane demonstrated, there are trends (in the same direction) in allele frequencies in both DN and DR complications alone. Thus, the combined phenotype (capturing both these trends simultaneously) should show more extreme differences. This difference should be detectable by also using a smaller patient sample, suggesting one explanation for why the association was seen with the combined phenotype (regardless of the smaller patient sample) and not with the DR phenotype alone.

This study was based on the hypothesis that genetic variation in specific thiamine transporters affects the capability of achieving sufficiently high intracellular thiamine levels. Thus, only four genes of interest were studied. We have previously performed GWAS on DN in FinnDiane (5), and therefore, setting the significance threshold for a candidate gene study in the GWAS setting is not straightforward. Nevertheless, after replication, the detected associations reached genome-wide significance. The resulting ORs were high compared with those of many other complex traits, but this might reflect the extreme phenotype setting used. Although in type 1 diabetes most

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. People having this extreme phenotype are rare because showing only no/minimal DR/DN after 20 years of diabetes is uncommon.

The associated SNPs are located in the intronic regions of *SLC19A3* (rs6713116 in the first and rs12694743 in the fourth intron of the ENST00000258403 transcript), but their functional mechanism remains unknown. One possibility is that these variants affect regulatory protein binding in the region and the expression of *SLC19A3*. However, we could not find any functional annotation or regulatory elements underlying the associated SNPs affecting hTHTR2 or its expression in the publicly available databases. Therefore, these SNPs possibly represent an association of another nearby variant in LD with them, and possibly even in another gene not genotyped or imputed in the present data. Nevertheless, public databases support the importance of *SLC19A3* in DN. For instance, data by Schmid et al. (31) available in the Nephromine database (www.nephromine.org) showed that *SLC19A3* is underexpressed in DN in the kidney ($P = 0.016$, fold change = −1.14, rank 1,720 of 12,624). Elucidating the true mechanisms, however, requires additional functional studies.

SLC19A3 encodes hTHTR2, which is expressed mainly in the intestine, liver, kidney, and placenta. It is absent from marrow stem cells, pancreatic β-cells, and cochlear hairy cells of the inner ear and is probably involved above all in intestinal thiamine absorption (32–34). Mutations in *SLC19A3* are known to cause biotine-responsive basal ganglial disease (Online Mendelian Inheritance in Man [OMIM] 607483) (35) and thiamine-responsive encephalopathy (OMIM 606152) (36). However, those mutations produce truncated proteins, whereas the SNPs described in this article may result in more subtle functional changes of the thiamine transporter hTHTR2, with biological importance in the development of both DR and DN. Thiamine acts as a coenzyme for three key enzymes in glucose metabolism. Transketolase shifts glyceraldehyde 3-phosphate, which is particularly active in glycation of proteins and advanced glycation end-product formation, from glycolysis into the pentose phosphate shunt. Pyruvate dehydrogenase converts pyruvate, the final product of glycolysis, into acetyl-CoA, which then enters the Krebs cycle. α-Ketoglutarate dehydrogenase catalyzes the oxidation of α-ketoglutaric acid to succinyl-CoA 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 different regions involved in related processes may exist. In addition, without performing functional analyses, we cannot comment on the mechanisms through which the SNPs affect the gene. Strengths of this study include the large discovery cohort with well-defined phenotypes. On top of this, the finding for the combined phenotype was replicated in one of the available

Table 4—Significant results of association analyses with the combined phenotype of DR and ESRD in the FinnDiane cohort, replication results in the WESDR cohort, and results of meta-analyses

chr	SNP	bp	Allele		Cohort	Imputation quality	Participants (*n*)			MAF			OR* (95% CI)	*P* value
			Minor	Major			Case	Control	Total	Case	Control	All		
2	rs12694743	228,266,664	G	A	FinnDiane: basic†	0.93	369	190	559	0.12	0.23	0.16	0.31 (0.20–0.47)	7.51×10^{-8}
					FinnDiane: adjusted‡	0.93	298	190	488	0.12	0.23	0.16	0.28 (0.17–0.45)	1.29×10^{-7}
					WESDR: basic§	0.96	82	112	194	0.06	0.11	0.09	0.34 (0.13–0.91)	0.033
					WESDR: adjusted‖	0.96	82	112	194	0.06	0.11	0.09	0.29 (0.08–1.10)	0.068
					Meta-analysis: basic	—	451	302	753	0.11	0.18	0.14	0.32 (0.22–0.47)	7.14×10^{-9}
					Meta-analysis: adjusted	—	380	302	682	0.11	0.18	0.14	0.28 (0.18–0.44)	2.30×10^{-8}
2	rs6713116	228,275,342	T	C	FinnDiane: basic†	0.81	369	190	559	0.14	0.22	0.17	0.26 (0.15–0.44)	7.49×10^{-7}
					FinnDiane: adjusted‡	0.81	298	190	488	0.14	0.22	0.17	0.23 (0.13–0.42)	1.90×10^{-6}
					WESDR: basic§	1	82	112	194	0.08	0.15	0.12	0.39 (0.16–0.92)	0.032
					WESDR: adjusted‖	1	82	112	194	0.08	0.15	0.12	0.51 (0.17–1.50)	0.219
					Meta-analysis: basic	—	451	302	753	0.13	0.19	0.15	0.29 (0.18–0.46)	9.62×10^{-8}
					Meta-analysis: adjusted	—	380	302	682	0.13	0.19	0.16	0.28 (0.17–0.47)	1.90×10^{-6}

chr, chromosome; bp, base pair; MAF, minor allele frequency. *OR of logistic regression for each copy of minor allele. †Model adjusted for age, sex, diabetes duration, and first 10 genetic PCs. ‡Model adjusted for age, sex, diabetes duration, first 10 genetic PCs, log(BMI), and HbA_{1c}. §Model adjusted for age, sex, duration of diabetes, and first 10 PCs. ‖Model adjusted for age, sex, duration of diabetes, first 10 PCs, mean HbA_{1c} during WESDR, and BMI.

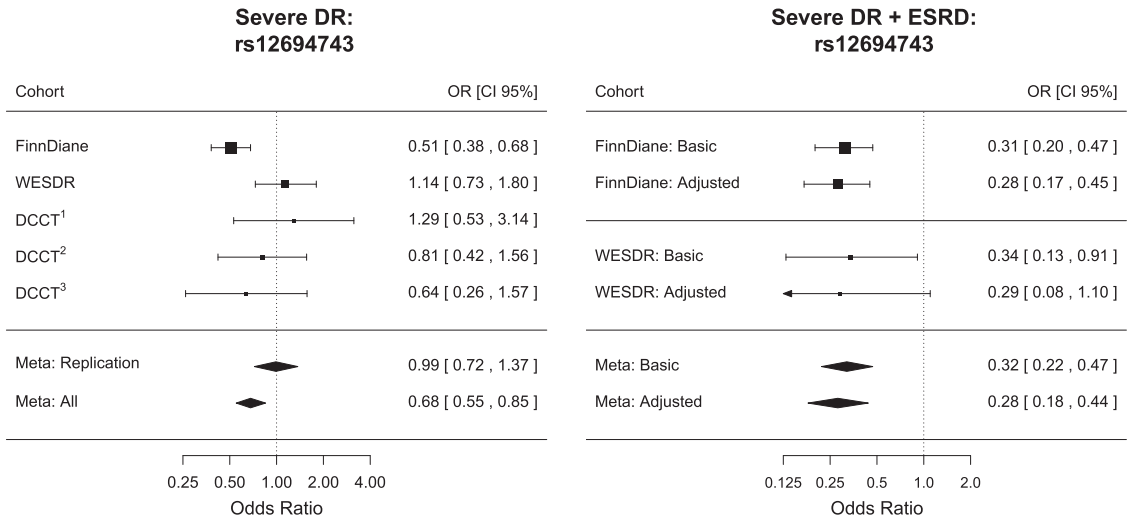

Figure 1—Forest plots of replication results and meta-analyses for lead SNPs in the analysis of DR and the combined phenotype. The point size of the diamonds and the squares are proportional to the precision of the estimates of OR. Results for rs6713116 behaved similarly. DCCT[1], DCCT primary cohort; DCCT[2], DCCT secondary cohort–conventional treatment; DCCT[3], DCCT secondary cohort–intensive treatment.

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.

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