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RESEARCH ARTICLE

Association of transketolase polymorphisms with measures of polyneuropathy in patients with recently diagnosed diabetes

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Abstract

Background Shunting of glycolytic intermediates into the pentose phosphate pathway has been suggested to protect from hyperglycaemia-induced microvascular damage. We hypothesized that genetic variability in the gene encoding transketolase, a key pentose phosphate pathway enzyme, contributes to early nerve dysfunction in recent-onset diabetes.

Methods In this cross-sectional study, we assessed nine single nucleotide polymorphisms (SNPs) in the transketolase gene, plasma methylglyoxal concentrations, and clinical and quantitative measures of peripheral nerve function in 165 type 1 and 373 type 2 diabetic patients with a diabetes duration up to 1 year.

Results The Total Symptom Score was associated with transketolase SNPs rs7648309, rs62255988, and rs7633966, while peroneal motor nerve conduction velocity (MNCV) correlated only with rs7648309 ($P < 0.01$). Cold thermal detection threshold (TDT) (foot) was associated with transketolase SNPs rs11130362 and rs7648309, while warm TDT (hand) correlated with rs62255988 and rs7648309 ($P < 0.01$). After Bonferroni correction, the correlations of transketolase SNP rs7648309 with Total Symptom Score and rs62255988 with warm TDT (hand) remained statistically significant. Among subgroups, men with type 2 diabetes showed the strongest associations. No associations were observed between each of the nine tagged transketolase SNPs and plasma methylglyoxal concentrations.

Conclusions The observed associations of genetic variation in transketolase enzyme with neuropathic symptoms and reduced thermal sensation in recent-onset diabetes suggest a role of pathways metabolizing glycolytic intermediates in early diabetic neuropathy. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords diabetic polyneuropathy; neuropathic symptoms; thermal detection thresholds; nerve conduction velocity; transketolase; single nucleotide polymorphisms

Abbreviations AGE, Advanced glycation end-product; FN3K, Fructosamine 3-kinase; DSPN, Diabetic sensorimotor polyneuropathy; GDS, German Diabetes Study; GAPDH, Glyceraldehyde phosphate dehydrogenase; MAF, Minor allele frequency; MNCV, Motor nerve conduction velocity; NDS, Neuropathy Disability Score; NSS, Neuropathy Symptom Score; PPP, Pentose phosphate pathway; RAGE, Receptor for advanced glycation

end-products; SNAP, Sensory nerve action potential; SNCV, Sensory nerve conduction velocity; SNP, Single nucleotide polymorphism; TDT, Thermal detection threshold; TDP, Thiamine diphosphate; TSS, Total Symptom Score; TKTL-1, Transketolase-like 1; VPT, Vibration perception threshold

Introduction

Diabetic sensorimotor polyneuropathy (DSPN) is a serious complication of diabetes encountered in about one-third of all diabetic patients [1] and predictor of feared endpoints such as diabetic foot ulcers [2], cardiovascular morbidity [3], and mortality [4]. Four major molecular pathways have been implicated in glucose-mediated microvascular complications triggered by a single hyperglycaemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain [5]. Superoxide overproduction partially inhibits the glycolytic enzyme glyceraldehyde phosphate dehydrogenase (GAPDH), thereby diverting upstream metabolites from glycolysis into glucose-driven signalling pathways of glucose overuse. Fructose-6-phosphate and glyceraldehyde-3-phosphate are two of these upstream metabolites which are also end products of the non-oxidative branch of the pentose phosphate pathway (PPP) [6]. These metabolites are generated by the thiamine diphosphate-dependent enzyme transketolase that catalyses several key reactions of the nonoxidative branch of the PPP and serves as a bridge between the oxidative part of PPP and oxidative decarboxyl metabolism of glucose, allowing the cell to adapt to a variety of metabolic needs under different environmental conditions [7]. Under physiological conditions, transketolase enzyme activity operates approximately proportional to substrate concentration [8]. Hyperglycaemia-induced rise in fructose-6-phosphate and glyceraldehyde-3-phosphate concentrations diverts these metabolites to pentose-5-phosphates and erythrose-4-phosphate under conditions where transketolase is fully activated by its cofactor thiamine [6]. Indeed, transketolase activation in retinas of diabetic animals inhibited three of the four major biochemical pathways implicated in the pathogenesis of hyperglycaemia-induced microvascular damage and prevented experimental diabetic retinopathy [6]. Thus, transketolase activation may limit the turnover of damaging pathways by reducing the availability of their precursors [9].

Evidence has accumulated suggesting that thiamine metabolism is altered in diabetes, but it remains unclear which mechanisms contribute most to the deficient action of thiamine [10]. Patients with type 1 and type 2 diabetes frequently showed low plasma thiamine concentrations in association with increased thiamine clearance, while erythrocyte transketolase activity was not reduced but correlated inversely with urinary albumin excretion [11]. In contrast, a decrease in basal erythrocyte transketolase activity without evidence of thiamine deficiency was observed in diabetic patients with polyneuropathy [12]. Early work suggested that transketolase activity was inhibited by plasma, cerebrospinal fluid, and low molecular weight dialysate fractions obtained from patients with uremic neuropathy. Data of clinical grading of the neurological deficits and values of motor nerve conduction velocity revealed a correlation between the extent of uremic neuropathy and the degree of nervous tissue transketolase inhibition [13].

In a recent study, progression of nephropathy and incidence of major cardiovascular events were associated with genetic variability in the transketolase enzyme in diabetic patients [9], but no data addressing such a possible relationship to diabetic neuropathy is available. Because shunting of glycolytic intermediates into the PPP has been suggested to protect from hyperglycaemia-induced microvascular damage, we hypothesized that genetic variability in transketolase, one of the key PPP enzymes, could contribute to early nerve dysfunction in individuals with recent-onset diabetes.

Materials and methods

The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Heinrich Heine University, Düsseldorf, Germany. All participants provided a written informed consent. Included were 538 participants of the German Diabetes Study (GDS) baseline cohort, 165 and 373 of whom had type 1 and type 2 diabetes, respectively. The GDS is a long-term prospective observational study assessing the long-term course of diabetes and its sequelae (ClinicalTrials.gov Identifier: NCT01055093) [14]. Inclusion criteria for entry into the GDS are type 1 or type 2 diabetes, known diabetes duration of $\leq$1 year and age of 18–69 years at baseline assessment. Exclusion criteria for the present study were type 3 diabetes, pregnancy, severe diseases (e.g. cancer), psychiatric disorders, immunosuppressive therapy, limited cooperation ability, and neuropathy from causes other than diabetes.

Single nucleotide polymorphisms (SNPs)

Genomic DNA was extracted from whole blood using the blood extraction kit (Qiagen, Hilden, Germany). Selection

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of tagging SNPs was based on publicly available data of the International 1000 genomes Project derived from Utah residents with Central European ancestry http://1000genomes.org. We screened in silico the complete transketolase gene spanning 53258723–53290130 (chromosome assembly GRCh37.p13) on chromosome 3. We found 76 SNPs with a minor allele frequency (MAF) ≥0.2. Among these, 13 tagging SNPs were selected for genotyping using tagger software from haploview freeware (https://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview). Genotyping was successful for nine of these SNPs. The nine tagging SNPs covered 73% of all common genetic variation in the locus (MAF ≥0.2). The nine transketolase tagging SNPs were genotyped using the Sequenom massARRAY system with iPLEX software (Sequenom, Hamburg, Germany) as previously described [15]. The genotyping success rates were 99.7%. The Sequenom results were validated by bidirectional sequencing in 50 randomly selected subjects, and both methods gave 100% identical results ($r = 1.00$).

Neurophysiological and clinical measures

Peripheral nerve function tests were performed as previously described [16]. In brief, motor nerve conduction velocity (NCV) was measured in the median, ulnar, and peroneal nerves, while sensory NCV and sensory nerve action potentials (SNAP) were determined in the median, ulnar, and sural nerves at a skin temperature of 33–34 °C using surface electrodes (Nicolet VikingQuest, Natus Medical, San Carlos, CA). Quantitative sensory testing included measurement of the vibration perception threshold (VPT) at the second metacarpal bone and medial malleolus using the method of limits (Vibrameter, Somedic, Stockholm, Sweden) and thermal detection thresholds (TDT) including warm and cold thresholds at the thenar eminence and dorsum of the foot using the method of limits (TSA-II NeuroSensory Analyzer, Medoc, Ramat Yishai, Israel). Neurological examination was performed using the Neuropathy Disability Score (NDS) and Neuropathy Symptom Score (NSS) [17]. DSPN was defined according to modified Toronto Consensus criteria [18] including subclinical DSPN (stage 1a), asymptomatic DSPN with neuropathic signs but without neuropathic symptoms (stage 1b), and symptomatic DSPN with neuropathic symptoms with or without neuropathic signs (stage 2). Confirmed DSPN was defined by the presence of ≥2 abnormal nerve conduction attributes <2.5th percentile with ≥1 being peroneal MNCV, sural SNCV, or sural SNAP.

Plasma methylglyoxal was determined by HPLC after derivatization with 1,2-diamino-4,5-dimethoxybenezene as previously described [19]. To prevent overestimation of MG levels by peroxidases and trace metals, derivatization was performed in the presence of sodium azide and DETAPAC as suggested previously [20].

Statistical analysis

Comparisons between type 1 and type 2 diabetes subjects were performed using the non-parametric *t*-test (Mann–Whitney U test) for continuous variables and Fisher's exact test or χ^2 test where appropriate for categorical variables. Associations of SNPs with measures of DSPN were studied by the Kruskal–Wallis test. Considering that 9 SNPs and 18 neurological measures were investigated, *P* values were adjusted for multiple comparisons using the Bonferroni correction. All statistical tests were performed two sided. The level of significance before and after Bonferroni correction was set at $\alpha = 0.05$ and $\alpha = 0.0003$ ($0.05/162 = 0.0003$), respectively. Statistical analyses were performed using IBM SPSS Statistics 22 software.

Results

The demographic and clinical characteristics of the two groups studied are listed in Table 1. Compared to type 1 diabetic patients, those with type 2 diabetes were older and showed higher BMI, waist circumference, systolic and diastolic blood pressure, triglycerides, LDL cholesterol, albuminuria, NSS, NDS, metacarpal and malleolar VPT, and TDT to warm and cold stimuli on the hand and foot as well as lower HDL cholesterol, HbA_{1c}, peroneal and median MNCV, median SNCV, and sural, median, and ulnar SNAP (all $P < 0.05$). No significant differences between the groups were observed for sex, heart rate, serum creatinine, known diabetes duration, percentages of smokers, TSS in the feet, ulnar MNCV, sural SNCV, and ulnar SNCV.

The allele distributions in the nine transketolase tagging SNPs are shown in Table 2. All SNPs were in Hardy–Weinberg Equilibrium. The associations of transketolase SNPs with neuropathic symptoms and neurophysiological parameters that achieved statistical significance are shown in Table 3. In the entire group before Bonferroni correction, TSS in the feet was associated with transketolase SNPs rs7648309, rs62255988, and rs7633966, while peroneal MNCV correlated with rs7648309 (all $P < 0.01$). Cold TDT (foot) was associated with transketolase SNPs rs11130362 and rs7648309, while warm TDT (hand) correlated with rs62255988 and rs7648309 (all $P < 0.01$). After Bonferroni correction, the correlations of transketolase SNP rs7648309 with TSS ($P = 0.024$) and rs62255988

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Table 1. Demographic, clinical, and neurophysiological characteristics of the subjects studied

	Type 1 diabetes	Type 2 diabetes
n	165	373
Sex (% male)†	60.0 (38.5–45.5)	65.1 (57.3–66.3)
Age (years)	35 (25–45)	54 (46–61)*
BMI (kg/m^2)	23.9 (21.9–26.7)	30.6 (27.0–35.2)*
Waist circumference (cm)	97 (92–104)	108 (101–116)*
Heart rate (bpm)	68 (62–76)	70 (63–77)
Systolic blood pressure (mmHg)	116 (109–126)	132 (121–143)*
Diastolic blood pressure (mmHg)	65 (59–71)	74 (68–81)*
Triglycerides (mg/dl)	68 (52–104)	129 (91–188)*
HDL Cholesterol (mg/dl)	58 (47–69)	47 (39–55)*
LDL cholesterol (mg/dl)	101 (84–122)	125 (102–152)*
Albuminuria (mg/l)	12.0 (12.0–12.0)	12.0 (12.0–14.0)*
Creatinine (mg/dl)	0.82 (0.73–0.94)	0.88 (0.77–0.98)
HbA_{1c} (%)	6.5 (6.0–7.4)	6.2 (5.8–6.9)*
HbA_{1c} (mmol/mol)	47.5 (41.5–57.4)	44.3 (39.9–51.9)*
Known diabetes duration (days)	175 (117–281)	177 (100–262)
Regular smokers (%)†	14.5 (11.3–16.2)	14.5 (8.3–14.2)
Neuropathy Symptom Score (points)‡	0.39 ± 1.44	1.09 ± 2.33*
Neuropathy Disability Score (points)‡	0.51 ± 1.15	1.64 ± 2.14*
Total Symptom Score feet (points)‡	0.10 ± 0.43	0.36 ± 1.37
Peroneal MNCV (m/s)	46.0 (44.0–48.8)	45.3 (42.0–49.0)*
Median MNCV (m/s)	55.3 (53.0–58.0)	53.4 (51.0–56.2)*
Ulnar MNCV (m/s)	57.0 (54.0–60.0)	56.0 (52.0–60.0)
Sural SNCV (m/s)	45.1 (43.0–49.0)	45.0 (41.0–48.3)
Median SNCV (m/s)	56.0 (51.6–59.0)	52.0 (48.0–56.5)*
Ulnar SNCV (m/s)	54.2 (51.0–57.0)	54.0 (50.0–57.1)
Sural SNAP (μV)	10.0 (6.5–15.1)	8.0 (4.9–11.7)*
Median SNAP (μV)	6.95 (4.72–9.64)	5.34 (3.45–7.61)*
Ulnar SNAP (μV)	6.44 (4.37–9.08)	4.45 (2.73–6.28)*
Metacarpal VPT (μm)	0.23 (0.15–0.34)	0.37 (0.22–0.59)*
Malleolar VPT (μm)	0.42 (0.28–0.71)	1.04 (0.50–2.32)*
Cold TDT hand (°C)	30.7 (30.3–31.0)	30.4 (29.9–30.8)*
Warm TDT hand (°C)	33.6 (33.2–33.9)	33.9 (33.4–34.6)*
Cold TDT foot (°C)	29.3 (27.5–30.3)	28.6 (26.0–30.1)*
Warm TDT foot (°C)	37.8 (35.8–41.0)	39.6 (36.6–43.4)*

*$P < 0.05$ vs type 1 diabetes; data are †percentages (95% confidence intervals), ‡mean ± SD or median (first to third quartiles).
MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential; VPT, vibration perception threshold; TDT, thermal detection threshold.

with warm TDT (hand) ($P = 0.049$) remained statistically significant. In the group with type 1 diabetes before Bonferroni correction, TSS in the feet was associated with transketolase SNP rs62255988, while peroneal MNCV correlated with rs7648309, rs9284890, rs12629312, and rs9820979 (all $P < 0.05$). Moreover, median MNCV was associated with rs9284890, while ulnar SNCV was linked with transketolase SNP rs12629312 and warm TDT (hand) to rs7648309 (all $P < 0.05$). In the group with type 2 diabetes before Bonferroni correction, TSS (foot) was associated with transketolase SNPs rs7648309, rs62255988, and rs7633966 (all $P < 0.05$). Furthermore, metacarpal VPT correlated with transketolase SNP rs9820979, while cold TDT (foot) was related to rs11130362 and rs7648309, and warm TDT (hand) correlated with rs7648309 and rs62255988 (all $P < 0.01$). After Bonferroni correction, the associations in each diabetes group were no longer statistically significant.

The *P* values for the associations of transketolase SNPs with the TSS and neurophysiological parameters separately according to sex are shown in Table 4. In the entire male group before Bonferroni correction, TSS in the feet was associated with transketolase SNPs rs7648309, rs62255988, and rs7633966, while peroneal MNCV correlated with rs7648309 (all $P < 0.01$). Cold TDT (foot) was associated with transketolase SNPs rs11130362 and rs7648309, while warm TDT (hand) correlated with rs62255988 and rs7648309 (all $P < 0.05$). After Bonferroni correction, the correlations between the TSS and transketolase SNP rs7648309 ($P = 0.013$) and rs62255988 ($P = 0.005$) with TSS remained statistically significant. In the entire female group before Bonferroni correction, only peroneal MNCV was associated with transketolase SNPs rs7648309 and rs9284890 ($P < 0.01$), but statistical significance was lost after Bonferroni correction. In the male type 1 diabetes group before Bonferroni correction, only peroneal MNCV

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Table 2. Genotype of the population studied

rs9284890		rs11130362		rs7648309		rs62255988		rs12629312		rs9820979		rs9879195		rs17030933		rs7633966	
	% (*n*)		% (*n*)		% (*n*)		% (*n*)		% (*n*)		% (*n*)		% (*n*)		% (*n*)		% (*n*)
TT	28.1 (142)	**CC**	57.5 (284)	**GG**	40.1 (208)	**GG**	38 (194)	**GG**	64.4 (328)	**GG**	59.5 (307)	**CC**	55.2 (288)	**GG**	60 (310)	**TT**	36.9 (191)
CT	49.6 (251)	**TC**	36 (178)	**GA**	45.1 (234)	**GA**	46.7 (238)	**GA**	32.4 (165)	**GA**	34.9 (180)	**CG**	37.7 (197)	**GC**	35.6 (184)	**TG**	47.7 (247)
CC	22.3 (113)	**TT**	6.5 (32)	**AA**	14.8 (77)	**AA**	15.3 (78)	**AA**	3.1 (16)	**AA**	5.6 (29)	**GG**	7.1 (37)	**CC**	4.4 (23)	**GG**	15.4 (80)
T	0.53	**C**	0.76	**G**	0.63	**G**	0.61	**G**	0.81	**G**	0.77	**C**	0.74	**G**	0.78	**T**	0.61
C	0.47	**T**	0.24	**A**	0.37	**A**	0.39	**A**	0.19	**A**	0.23	**G**	0.26	**C**	0.22	**G**	0.39
HWE																	
χ^2	0.01		0.33		0.71		0.13		0.76		0.15		0.17		0.43		0.00
ρ	2.99		2.90		2.92		2.96		3.37		2.92		2.93		3.20		3.00
ω	0.00		0.03		0.04		0.02		0.04		0.02		0.02		0.03		0.00
P	0.92		0.57		0.40		0.72		0.38		0.70		0.68		0.51		0.99

HWE, Hardy–Weinberg Equilibrium with χ^2; Chi squared (1 degree of freedom); ρ, Pearson; ω, estimated effect size; *P*, *p* value. All SNPs are in HWE.

correlated with transketolase SNP rs7648309 ($P = 0.041$), while in women with type 1 diabetes peroneal MNCV correlated with rs7648309, rs9284890, and rs9820979 (all $P < 0.05$) and ulnar SNCV was associated with rs12629312 ($P = 0.004$), but statistical significance was lost after Bonferroni correction. In the male group with type 2 diabetes before Bonferroni correction, TSS in the feet was associated with transketolase SNPs rs7648309, rs62255988, and rs7633966, while peroneal MNCV correlated with rs7648309 (all $P < 0.05$). Cold TDT (foot) was associated with transketolase SNPs rs11130362 and rs7648309, while warm TDT (hand) correlated with rs62255988 and rs7648309 and metacarpal VPT correlated with rs9820979 (all $P < 0.05$). After Bonferroni correction, the correlations between the TSS and transketolase SNP rs7648309 ($P = 0.036$) and rs62255988 ($P = 0.031$) with TSS remained statistically significant. In the female group with type 2 diabetes only peroneal MNCV and transketolase SNP rs9284890 were associated ($P = 0.029$), but statistical significance was lost after Bonferroni correction.

Supplementary Table 1 shows the percentages with 95% confidence intervals of patients with confirmed DSPN and abnormal neurophysiological tests (<2.5th or >97.5th percentile of control subjects) for the SNPs listed in Table 2. Peroneal MNCV correlated with transketolase SNP rs9284890, while warm TDT hand was associated with rs62255988 and DSPN correlated with rs9284890 (all $P < 0.05$), but statistical significance was lost after Bonferroni correction.

Table 5 shows the relationship between transketolase SNPs and plasma methylglyoxal concentrations. No associations were observed between each of the nine tagged transketolase SNPs and plasma methylglyoxal levels.

Discussion

The results of this study demonstrate associations of genetic variability in transketolase enzyme with neuropathic symptoms and reduced thermal detection threshold in recently diagnosed diabetic patients with known diabetes duration up to 1 year, suggesting a role of pathways metabolizing glycolytic intermediates in early diabetic nerve dysfunction. Subgroup analysis stratified by sex and diabetes type revealed that these associations were determined primarily by male sex and type 2 diabetes.

To our knowledge, genetic variability in transketolase enzyme has not been previously described in the context of diabetic polyneuropathy. Only two reports from a single institution have addressed the role of genetic variation in the transketolase enzyme in diabetic patients with various stages of nephropathy. The first report focused on

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Table 3. Associations of transketolase SNPs with neuropathic symptoms and neurophysiological parameters

		All subjects ($n = 538$)				Type 1 diabetes ($n = 165$)	Type 2 diabetes ($n = 373$)
	SNP	Genotype AA	Genotype Aa	Genotype aa	*P* value	*P* value	*P* value
TSS feet (points)	rs7648309	0.36 ± 1.30	0.07 ± 0.37	0.68 ± 2.00	**0.00015***	0.251	**0.001**
	rs62255988	0.35 ± 1.25	0.07 ± 0.39	0.63 ± 2.00	**0.001**	**0.022**	**0.014**
	rs7633966	0.22 ± 0.91	0.49 ± 1.66	0.03 ± 0.16	**0.004**	0.768	**0.003**
Peroneal MNCV (m/s)	rs7648309	44.7 ± 5.3	45.0 ± 4.4	44.3 ± 4.4	**0.008**	**0.028**	0.123
	rs9284890	45.0 ± 5.3	46.0 ± 4.2	44.4 ± 4.5	0.157	**0.003**	0.338
	rs12629312	44.9 ± 5.0	45.3 ± 4.7	45.9 ± 5.5	0.462	**0.006**	0.786
	rs9820979	44.9 ± 5.0	45.3 ± 4.8	46.5 ± 4.8	0.230	**0.009**	0.603
Median MNCV (m/s)	rs9284890	54.1 ± 4.2	54.4 ± 4.2	53.4 ± 4.5	0.410	**0.006**	0.993
Ulnar SNCV (m/s)	rs12629312	53.3 ± 5.3	54.0 ± 5.5	55.4 ± 4.4	0.154	**0.003**	0.284
Metacarpal VPT (μm)	rs9820979	0.41 ± 0.41	0.45 ± 0.37	0.37 ± 0.31	0.181	0.505	**0.002**
Cold TDT foot (°C)	rs11130362	27.5 ± 4.2	27.3 ± 5.0	29.4 ± 2.1	**0.002**	0.224	**0.008**
	rs7648309	27.2 ± 4.6	28.1 ± 4.2	27.1 ± 4.8	**0.002**	0.443	**0.005**
Warm TDT hand (°C)	rs7648309	34.2 ± 1.5	34.0 ± 1.4	34.3 ± 1.4	**0.003**	0.191	**0.009**
	rs62255988	34.3 ± 1.5	33.9 ± 1.4	34.2 ± 1.3	**0.0003****	**0.010**	**0.006**

Values in bold indicate differences between groups ($P < 0.05$; Kruskal-Wallis test).
*$P = 0.024$, and **$P = 0.049$ after Bonferroni correction.
TSS, Total Symptom Score; MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; VPT, vibration perception threshold; TDT, thermal detection threshold.

concentrations of thiamine and its esters in plasma and whole blood and transketolase-catalysed reaction together with 14 tagging SNPs in the transketolase, transaldolase, and transketolase-like 1 (TKTL-1) genes. Among 240 subjects studied with variable degrees of nephropathy, 69 had type 1 diabetes, 4 had latent autoimmune diabetes in adults (LADA), and 167 had type 2 diabetes. Among 10 tagging SNPs in the transketolase gene, there were no differences in allelic, genotype, or haplotype distributions between groups of diabetic patients with and without nephropathy. Moreover, no associations were found between genotypes of all 10 SNPs in the transketolase locus and transketolase activity in erythrocytes [21]. In the second report from the same institution, 19 SNPs in six genes encoding enzymes metabolizing glycolytic intermediates produced in excess under hyperglycaemic conditions (transketolase, transaldolase, TKTL-1, fructosamine 3-kinase (FN3K), glyoxalase 1, and glucose-6-phosphate dehydrogenase) were analysed in 314 type 2 diabetic subjects with variable stages of nephropathy who were followed up for a median of 38 months. Progression of diabetic nephropathy was

Table 4. *P* values for gender-specific associations of transketolase SNPs with neuropathic symptoms and neurophysiological parameters

		All subjects ($n = 538$)		Type 1 diabetes ($n = 165$)		Type 2 diabetes ($n = 373$)	
	SNP	Male ($n = 342$)	Female ($n = 196$)	Male ($n = 99$)	Female ($n = 66$)	Male ($n = 243$)	Female ($n = 130$)
TSS feet	rs7648309	**0.00008***	0.380	0.305	0.702	**0.00022**$^{+}$**	0.351
	rs62255988	**0.00003****	0.962	0.179	0.110	**0.00019**$^{++}$**	0.727
	rs7633966	**0.003**	0.312	0.785	0.680	**0.002**	0.396
Peroneal MNCV	rs7648309	**0.007**	**0.008**	**0.041**	**0.002**	**0.032**	0.408
	rs9284890	0.157	**0.002**	0.247	**0.002**	0.251	0.029
	rs12629312	0.509	0.912	0.070	0.086	0.973	0.416
	rs9820979	0.301	0.509	0.345	**0.013**	0.627	0.592
Median MNCV	rs9284890	0.410	0.704	0.086	0.059	0.906	0.589
Ulnar SNCV	rs12629312	0.756	0.059	0.326	**0.004**	0.995	0.120
Metacarpal VPT	rs9820979	0.204	0.620	0.426	0.696	**0.014**	0.185
Cold TDT foot	rs11130362	**0.005**	0.129	0.189	0.097	**0.016**	0.396
	rs7648309	**0.015**	0.127	0.387	0.931	**0.041**	0.084
Warm TDT hand	rs7648309	**0.020**	0.154	0.689	0.151	**0.025**	0.184
	rs62255988	**0.002**	0.122	0.157	0.066	**0.005**	0.650

Values in bold indicate differences between groups ($P < 0.05$; Kruskal-Wallis test).
*$P = 0.013$, **$P = 0.005$, $^{+}P = 0.036$, and $^{++}P = 0.031$ after Bonferroni correction.
TSS, Total Symptom Score; MNCV, motor nerve conduction velocity; SNCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential; VPT, vibration perception threshold; TDT, thermal detection threshold.

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Table 5. Relationship between transketolase SNPs and plasma methylglyoxal concentrations

	All subjects (n = 535)				Type 1 diabetes (n = 165)	Type 2 diabetes (n = 370)
SNP (allele)	Genotype AA MG (nmol/l)	Genotype Aa MG (nmol/l)	Genotype aa MG (nmol/l)	P value	P value	P value
rs9284890	396 ± 160	416 ± 149	399 ± 154	0.532	0.883	0.347
rs11130362	392 ± 156	417 ± 152	380 ± 154	0.340	0.562	0.562
rs7648309	399 ± 160	405 ± 156	422 ± 152	0.476	0.813	0.574
rs62255988	405 ± 157	396 ± 156	406 ± 145	0.779	0.798	0.522
rs12629312	404 ± 145	400 ± 169	422 ± 148	0.663	0.283	0.648
rs9820979	406 ± 147	398 ± 166	415 ± 176	0.708	0.099	0.681
rs9879195	410 ± 166	398 ± 150	417 ± 117	0.586	0.423	0.947
rs17030933	409 ± 157	401 ± 160	381 ± 106	0.660	0.987	0.552
rs7633966	408 ± 155	401 ± 166	397 ± 141	0.807	0.563	0.703

Data are mean ± SD; MG, methylglyoxal.

predicted by a combination of transketolase SNP rs11130362 and FN3K SNP rs1056534. In addition, transketolase SNP rs3736156 alone and also in combination with the previous two SNPs predicted the incidence of major cardiovascular events. No such a predictive value was observed for the remaining SNPs [9]. Thus, among SNPs in genes encoding enzymes metabolizing glycolytic intermediates, primarily transketolase SNPs may have an impact on the development of micro- and macrovascular damage. Our results add to the current knowledge by linking clinical and neurophysiological measures of polyneuropathy such as neuropathic symptoms and reduced thermal sensation at an early stage of diabetes to differences between genotypes of transketolase SNPs rs7648309 and rs62255988. Moreover, we found that differences between genotypes of these SNPs were associated with neuropathic symptoms preferentially in male individuals with type 2 diabetes.

Evidence has emerged to suggest that diabetic microvascular complications including neuropathy are caused by activation of four major pathways comprising the polyol pathway, advanced glycation end-product (AGE) formation with subsequent activation of the receptor for AGEs (RAGE) [22] and nuclear factor κB [23], protein kinase C (PKC) isoforms, and hexosamine pathway by mitochondrial overproduction of superoxide [5]. Transketolase can limit the activation of these pathways by lowering availability of their precursors [6]. Cells in general are capable of either decreasing oxidative stress by enzymatic and non-enzymatic antioxidant mechanisms and/or eliminating of damaging metabolites and their substrates (generated by overloaded glycolysis) that accumulate within cells. Such an alternative pathway for glucose oxidation is PPP which fulfils three important functions: first, production of reducing equivalent NADPH necessary for reduction of oxidized glutathione thus supporting intracellular antioxidant defence; second, production of ribose-5-phosphate required for the synthesis of nucleotides; and third, metabolic use of dietary pentoses. PPP consists of two branches: an irreversible oxidative branch necessary for NADPH and production of pentose phosphates and a reversible non-oxidative branch in which interconversion of three to seven carbons containing sugars occurs [7,8,10]. Transketolase, one of the key enzymes of the non-oxidative branch of PPP, transports 2-carbon units and catalyses formation of ribose-5-phosphate from glycolytic intermediates. Transketolase is activated by its cofactor thiamine and its derivative benfotiamine which has been shown to prevent experimental diabetic retinopathy by blocking the aforementioned major pathways responsible for hyperglycaemia-induced microvascular damage via the regulation of PPP resulting in diminished accumulation of triosephosphates and fructose-6-phosphate [6,10,24]. Several lines of evidence point to deficient action of thiamine in diabetes, but the mechanisms responsible for this finding remain currently unclear.

An enhanced metabolic flux leads to an increase in reactive carbonyl species via accumulation of triosephosphate intermediates. Methylglyoxal pertains to a class of reactive carbonyl species known as the alpha-oxoaldehydes or dicarbonyls. It is formed from the spontaneous degradation of triosephosphate intermediates and is a highly potent glycating agent [25,26]. Dicarbonyls such as methylglyoxal modify DNA and react with amino groups of intracellular and extracellular proteins to form advanced glycation end products (AGEs). Increased formation of AGEs results in enhanced cellular stress, dysfunction and ultimately cell death. The interaction of AGE-modified proteins through cell surface receptors, such as RAGE, can lead to increased cellular activation and sustained inflammatory responses [27]. In diabetic subjects the concentrations of intracellular triosephosphate intermediates and methylglyoxal in erythrocytes are excessively elevated as compared to healthy controls [26]. In experimental diabetic

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neuropathy, methylglyoxal reduces NCV, depolarizes sensory neurons, and induces post-translational modifications of the voltage-gated sodium channel $Nav_{1.8}$ which facilitate firing of nociceptive neurons resulting in thermal and mechanical hyperalgesia [28]. These findings suggest that the direct and indirect effects of dicarbonyls on nerves or neuronal microvascular network provide a unifying mechanism for the development and progression of diabetic neuropathy [27]. Here we hypothesized that, if genetic variability in the transketolase enzyme would slow down the turnover of PPP, this could be linked to an increased flux through the AGE pathway. Thus, we would have expected that transketolase SNPs are related to elevated methylglyoxal levels. However, our results do not support this notion, because we found no associations between each of the nine tagged transketolase SNPs and plasma methylglyoxal concentrations. One possible explanation for the lack of such an association could be that plasma methylglyoxal levels do not readily reflect intracellular methylglyoxal metabolism. In this context, it could be useful to determine cytosolic methylglyoxal concentrations and glyoxalase 1 activity, e.g. in mononuclear cells, to further address our hypothesis.

The present study has several limitations. First, we did not measure parameters of thiamine metabolism to explore a possible genotype–phenotype interaction between the latter and transketolase SNPs in relation to diabetic neuropathy. The two main tests routinely used for the assessment of thiamine status are the measurement of erythrocyte transketolase activity and the so-called thiamine effect. The former is measured by a kinetic reaction without adding thiamine. The thiamine effect has been defined as an increase of transketolase activity $\leq$15% after addition of a saturating amount of thiamine diphosphate (TDP) to the reaction [10]. However, neither erythrocyte transketolase activity nor the thiamine effect were altered in subjects with diabetes compared to controls or in diabetic individuals with vs those without microalbuminuria [11]. In contrast, low plasma thiamine concentrations were relatively frequent in patients with type 1 and type 2 diabetes presumably because of an increased thiamine clearance [11]. However, it has been argued that plasma thiamine predominantly reflects thiamine intake rather than cellular levels [10]. Second, the cross-sectional study design does not allow to determine the predictive value of transketolase SNPs on the development and progression of diabetic neuropathy which has to be verified in long-term prospective studies. The strengths of this work are the relatively large, homogeneous, and extensively phenotyped early-onset diabetes groups and comprehensive quantitative assessment of peripheral nerve function.

In conclusion, this study demonstrates associations of genetic variability in transketolase, a rate-limiting enzyme of pathways proposed to confer hypothetical protection against hyperglycaemia, with neuropathic symptoms and reduced thermal sensation in recently diagnosed diabetes. These associations were mainly driven by male sex and type 2 diabetes. Treatment with thiamine and its derivatives has been shown to augment PPP activity [10], normalize biomarkers of pathways causing microvascular complications in patients with type 1 diabetes [29], ameliorate neuropathic symptoms [30–32], and improve NCV in one study [33], but not in another [34], albeit the latter trial has been criticized for using a questionable study design [35]. Thus, pharmacogenomics considering transketolase SNPs could be useful to optimize such treatments in the future.

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Duality of interest

All authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

DZ designed the study and wrote the manuscript. DZ, ES, AS, BK, TF, NP, JS, and KM researched data, ES, AS, BK, TF, NP, JS, KM, PN, H-UH, HA-H, and MR revised the manuscript. All authors contributed substantially to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; and to drafting the work or revising it critically for important intellectual content; gave final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

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appropriately investigated and resolved. DZ is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website.

References

1. Ziegler D, Papanas N, Vinik AI, Shaw JE. Epidemiology of polyneuropathy in diabetes and prediabetes. *Handb Clin Neurol* 2014; **126**: 3–22.
2. Abbott CA, Vileikyte L, Williamson S, Carrington AL, Boulton AJM. Multicenter study of the incidence of and predictive risk factors for diabetic neuropathic foot ulceration. *Diabetes Care* 1998; **21**(7): 1071–1075.
3. Brownrigg JR, de Lusignan S, McGovern A, *et al.* Peripheral neuropathy and the risk of cardiovascular events in type 2 diabetes mellitus. *Heart* 2014; **100**(23): 1837–1843.
4. Calles-Escandón J, Lovato LC, Simons-Morton DG, *et al.* Effect of intensive compared with standard glycemia treatment strategies on mortality by baseline subgroup characteristics: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Diabetes Care* 2010; **33**(4): 721–727.
5. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001; **414**(6865): 813–820.
6. Hammes HP, Du X, Edelstein D, *et al.* Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 2003; **9**(3): 294–299.
7. Zhao J, Zhong CJ. A review on research progress of transketolase. *Neurosci Bull* 2009; **25**(2): 94–99.
8. Schenk G, Duggleby RG, Nixon PF. Properties and functions of the thiamin diphosphate dependent enzyme transketolase. *Int J Biochem Cell Biol* 1998; **30**(12): 1297–1318.
9. Tanhäuserová V, Kuricová K, Pácal L, *et al.* Genetic variability in enzymes of metabolic pathways conferring protection against non-enzymatic glycation versus diabetes-related morbidity and mortality. *Clin Chem Lab Med* 2014; **52**(1): 77–83.
10. Pácal L, Kuricová K, Kaňková K. Evidence for altered thiamine metabolism in diabetes: is there a potential to oppose gluco- and lipotoxicity by rational supplementation? *World J Diabetes* 2014; **5**(3): 288–295.
11. Thornalley PJ, Babaei-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**(10): 2164–2170.
12. Michalak S, Michałowska-Wender G, Adamcewicz G, Wender MB. Erythrocyte transketolase activity in patients with diabetic and alcoholic neuropathies. *Folia Neuropathol* 2013; **51**(3): 222–226.
13. Sterzel RB, Semar M, Lonergan ET, Treser G, Lange K. Relationship of nervous tissue transketolase to the neuropathy in chronic uremia. *J Clin Invest* 1971; **50**(11): 2295–2304.
14. Szendroedi J, Saxena A, Weber KS, *et al.* Cohort profile: the German Diabetes Study (GDS). *Cardiovasc Diabetol* 2016; **15**(1): 59. doi:10.1186/s12933-016-0374-9.
15. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr Protoc Hum Genet* 2009 Chapter 2: Unit 2.12. doi:10.1002/0471142905.hg0212s60.
16. Ziegler D, Siekierka-Kleiser E, Meyer B, Schweers M. Validation of a novel screening device (NeuroQuick) for quantitative assessment of small nerve fiber dysfunction as an early feature of diabetic polyneuropathy. *Diabetes Care* 2005; **28**(5): 1169–1174.
17. Young MJ, Boulton AJ, MacLeod AF, Williams DR, Sonksen PH. A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. *Diabetologia* 1993; **36**(2): 150–154.
18. Dyck PJ, Albers JW, Andersen H, *et al.* Diabetic polyneuropathies: update on research definition, diagnostic criteria and estimation of severity. *Diabetes Metab Res Rev* 2011; **27**(7): 620–628.
19. McLellan AC, Phillips SA, Thornalley PJ. The assay of methylglyoxal in biological systems by derivatization with 1,2-diamino-4,5-dimethoxybenzene. *Anal Biochem* 1992; **206**(1): 17–23.
20. Thornalley PJ, Rabbani N. Assay of methylglyoxal and glyoxal and control of peroxidase interference. *Biochem Soc Trans* 2014; **42**(2): 504–510.
21. Pácal L, Tomandl J, Svojanovsky J, *et al.* Role of thiamine status and genetic variability in transketolase and other pentose phosphate cycle enzymes in the progression of diabetic nephropathy. *Nephrol Dial Transplant* 2011; **26**(4): 1229–1236.
22. Haslbeck KM, Neundörfer B, Schlötzer-Schrehardtt U, *et al.* Activation of the RAGE pathway: a general mechanism in the pathogenesis of polyneuropathies? *Neurol Res* 2007; **29**(1): 103–110.
23. Bierhaus A, Haslbeck KM, Humpert PM, *et al.* Loss of pain perception in diabetes is dependent on a receptor of the immunoglobulin superfamily. *J Clin Invest* 2004; **114**(12): 1741–1751.
24. Rabbani N, Thornalley PJ. Emerging role of thiamine therapy for prevention and treatment of early-stage diabetic nephropathy. *Diabetes Obes Metab* 2011; **13**(7): 577–583.
25. Richard JP. Mechanism for the formation of methylglyoxal from triosephosphates. *Biochem Soc Trans* 1993; **21**(2): 549–553.
26. Fleming T, Cuny J, Nawroth G, *et al.* Is diabetes an acquired disorder of reactive glucose metabolites and their intermediates? *Diabetologia* 2012; **55**(4): 1151–1155.
27. Hidmark A, Fleming T, Vittas S, *et al.* A new paradigm to understand and treat diabetic neuropathy. *Exp Clin Endocrinol Diabetes* 2014; **122**(4): 201–207.
28. Bierhaus A, Fleming T, Stoyanov S, *et al.* Methylglyoxal modification of Na(v)1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nat Med* 2012; **18**(6): 926–933.
29. Du X, Edelstein D, Brownlee M. Oral benfotiamine plus alpha-lipoic acid normalises complication-causing pathways in type 1 diabetes. *Diabetologia* 2008; **51**(10): 1930–1932.
30. Abbas ZG, Swai AB. Evaluation of the efficacy of thiamine and pyridoxine in the treatment of symptomatic diabetic peripheral neuropathy. *East Afr Med J* 1997; **74**(12): 803–808.
31. Haupt E, Ledermann H, Köpcke W. Benfotiamine in the treatment of diabetic polyneuropathy—a three-week randomized, controlled pilot study (BEDIP study). *Int J Clin Pharmacol Ther* 2005; **43**(2): 71–77.
32. Stracke H, Gaus W, Achenbach U, Federlin K, Bretzel RG. Benfotiamine in diabetic polyneuropathy (BENDIP): results of a randomised, double blind, placebo-controlled clinical study. *Exp Clin Endocrinol Diabetes* 2008; **116**(10): 600–605.
33. Stracke H, Lindemann A, Federlin K. A benfotiamine-vitamin B combination in treatment of diabetic polyneuropathy.

Exp Clin Endocrinol Diabetes 1996; **104** (4): 311–316.
34. Fraser DA, Diep LM, Hovden IA, *et al.* The effects of long-term oral benfotiamine supplementation on peripheral nerve function and inflammatory markers in patients with type 1 diabetes: a 24-month, double-blind, randomized, placebo-controlled trial. *Diabetes Care* 2012; **35**(5): 1095–1097.
35. Ziegler D, Tesfaye S, Kempler P. Comment on: Fraser et al. The effects of long-term oral benfotiamine supplementation on peripheral nerve function and inflammatory markers in patients with type 1 diabetes: a 24-month, double-blind, randomized, placebo-controlled trial. *Diabetes Care* 2012; **35**: 1095–1097 Diabetes Care 2012; **35**(**5**): e79.

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