Genetic variation in thioredoxin interacting protein (TXNIP) is associated with hypertriglyceridaemia and blood pressure in diabetes mellitus

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Abstract

Aims Thioredoxin interacting protein (TXNIP) is an attractive candidate gene for diabetes or diabetic dyslipidaemia, since *TXNIP* is the strongest glucose-responsive gene in pancreatic B-cells, TXNIP deficiency in a mouse model is associated with hyperlipidaemia and *TXNIP* is located in the 1q21-1q23 chromosomal Type 2 diabetes mellitus (DM) locus. We set out to investigate whether metabolic effects of TXNIP that were previously reported in a murine model are also relevant in human Type 2 DM.

Methods The frequency distribution of a 3′ UTR single nucleotide polymorphism (SNP) in TXNIP was investigated in subjects with normal glucose tolerance (NGT; $n = 379$), impaired glucose tolerance (IGT; $n = 228$) and Type 2 DM ($n = 230$). Metabolic data were used to determine the effect of this SNP on parameters associated with lipid and glucose metabolism.

Results The frequency of the TXNIP variation did not differ between groups, but within the group of diabetic subjects, carriers of the TXNIP-T variant had 1.6-fold higher triglyceride concentrations ($P = 0.015$; $n = 136$) and a 5.5-mmHg higher diastolic blood pressure ($P = 0.02$; $n = 212$) than homozygous carriers of the common C-allele, whereas in non-diabetic subjects fasting glucose was 0.26 mmol/l lower ($P = 0.002$; $n = 478$) in carriers of the T-allele. Moreover, a significant interaction between plasma glucose concentrations and TXNIP polymorphism on plasma triglycerides was observed ($P = 0.012$; $n = 544$).

Conclusion This is the first report to implicate TXNIP in a human disorder of energy metabolism, Type 2 diabetes. The effect of TXNIP on triglycerides is influenced by plasma glucose concentrations, suggesting that the biological relevance of TXNIP variations may be particularly relevant in recurrent episodes of hyperglycaemia.

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Keywords diastolic blood pressure, genetic predisposition, thioredoxin interacting protein (TXNIP), triglyceride, Type 2 diabetes mellitus.

Abbreviations apo, apolipoprotein; BMI, body mass index; DBP, diastolic blood pressure; FCHL, familial combined hyperlipidaemia; GCNF, germ cell nuclear factor; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; PCR, polymerase chain reaction; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; T2DM, Type 2 diabetes mellitus; TRX, thioredoxin; TXNIP, thioredoxin interacting protein; USF-1, upstream stimulatory factor 1; VEGF, vascular endothelial growth factor

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Introduction

In several human populations, the 1q21-1q23 chromosomal area has been implicated in Type 2 diabetes mellitus (T2DM) [1] as well as in familial combined hyperlipidaemia (FCHL) [2]. In general, both T2DM and FCHL are believed to be determined by a disease-predisposing genetic background in concert with environmental risk factors such as obesity [3]. Moreover, subjects with T2DM and FCHL express common metabolic abnormalities, i.e. high plasma concentrations of fatty acids and/or triglycerides and insulin resistance. HcB19 mice are phenotypically characterized by inherited hyper-lipidaemia, especially hypertriglyceridaemia, and hepatic steatosis [4], two conditions that are common in both T2DM and FCHL [5,6]. Therefore, data obtained with this recently described mouse model of inherited hyperlipidaemia may be useful for gene discovery in these related human diseases.

The gene defect in these hyperlipidaemic HcB19 mice is a truncation in thioredoxin interacting protein (TXNIP) [7], which is located in a chromosomal region that is syntenic with the human 1q21-23 locus. *TXNIP* was initially proposed as a candidate gene for FCHL [7], but was later excluded for this disease in different populations [8–10]. More recently, upstream stimulatory factor 1 (*USF-1*) has been identified as the most likely causal gene for hyperlipidaemia in FCHL in this chromosomal locus [8]. Although the 1q21-23 locus is also implicated in T2DM and hypertriglyceridaemia is present in up to 50% of White T2DM patients [11], recent evidence strongly suggests that *USF-1* may not be a very relevant gene in diabetes [12,13]. For these and additional reasons (discussed below), we hypothesized that the *TXNIP* gene is involved in the development of hypertriglyceridaemia in diabetes.

Several indirect lines of evidence support the hypothesis that variations in TXNIP activity may affect an individual's susceptibility to development of diabetes. First, TXNIP regulates cellular thioredoxin (TRX) activity [14] and targeted expression of TRX in the pancreas protects B-cells against autoimmune- and streptozotocin-induced diabetes [15]. Second, Shalev *et al.* [16] have recently shown that *TXNIP* is the strongest glucose-responsive gene expressed in cultured human pancreatic β-cells. Lastly, increased insulin concentrations have been reported in TXNIP-deficient HcB19 mice [17], although this was not the case in the original HcB19 report [4]. These functional data on the roles of TXNIP and thioredoxin, combined with the chromosomal localization of TXNIP on 1q21-23 and the hypertriglyceridaemic effect of the truncation in HcB19 mice, support the hypothesis that variations in the *TXNIP* gene may affect susceptibility to T2DM or to hypertriglyceridaemia in T2DM.

It was the objective of the present study to analyse associations between a polymorphism in the 3′ UTR of the human *TXNIP* gene and several aspects of human diabetes in a population enriched in risk factors for T2DM. First, we established whether TXNIP variations could influence the risk of Type 2 diabetes in humans. Next, the effects of the TXNIP variant on

plasma lipid variables were determined in healthy subjects and diabetes patients. Finally, the effects of TXNIP variation on other phenotypic characteristics of Type 2 diabetes were analysed.

Methods

Study design

The hypothesis that the *TXNIP* gene is involved in the development of hypertriglyceridaemia in diabetes was investigated in the CODAM (COhortstudy on Diabetes and Atherosclerosis Maastricht; 574 subjects) population, extended with 270 subjects collected by the National Institute of Public Health (RIVM) according to exactly the same criteria [18,19]. Assignment of the normal glucose tolerance (NGT; $n = 379$), impaired glucose tolerance (IGT; $n = 228$) or T2DM ($n = 230$) status was done on the basis of an oral glucose tolerance test according to the 1999 World Health Organization criteria [20]. In the majority of the NGT, IGT and T2DM subjects, the following plasma parameters were determined using standard laboratory techniques [18]: apolipoprotein (apo) A and apoB, total and high-density lipoprotein cholesterol, triglycerides, glucose, insulin, free fatty acids, C-reactive protein. Urinary albumin concentrations and anthropomorphic markers such as waist circumference, height, weight, waist–hip ratio, body mass index (BMI) and blood pressure were also determined [21]. ApoA and apoB were determined in serum by auto analyser (Hitachi 912) using a Roche kit (Roche Diagnostics, Almere, the Netherlands). Homeostasis model assessment (HOMA-IR) was used to assess insulin resistance [22].

Participants provided informed consent and the studies were approved by local ethics committees. General characteristics of the groups with different glucose tolerance states are presented in Table 1.

Single nucleotide polymorphism analysis in TXNIP intron 7

TXNIP is a very small gene, spanning a genomic region of < 4000 base pairs. The 3′ UTR sequence of human TXNIP mRNA consists of 1307 nucleotides followed by a poly A tail (http://www.ensembl.org; v40, August 2006). The single nucleotide polymorphism (SNP) rs#7211 is located in the 3′ UTR, 402 base-pairs downstream of the TXNIP coding region (http://ncbi.nlm.nih.gov/SNP/). The C-allele (major allele) contains a restriction enzyme site for SnaB1 that is absent in the T-allele (minor allele). This particular SNP was chosen since it was a confirmed SNP with an acceptable reported average allele frequency of 25.5% T and 74.5% C (http://www.ncbi.nlm.nih.gov). DNA was prepared from peripheral blood mononuclear cells (QiaAmp DNA blood mini kit; Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification was performed using the following primer set: 5′TGTTTGTT-GGATGGG TTTAA-3′ (forward primer) and 5′CCACAT-GAAACCAACCA TC-3′ (backward primer). The amplification reactions contained 12.5 pmol of each primer, 0.75 U of Taq polymerase and 1.5 mm MgCl in a final volume of 12.5 μl. An annealing temperature of 57°C was used and the reaction was cycled for 35 repeats. Digestion of the PCR products with 5 U of SnaB1 (New England Biolabs, Ipswich, MA, USA) resulted

Table 1 General characteristics of the study population

| Glucose tolerance status | Non-diabetic subjects | | Diabetic subjects | ANOVA/Kruskal–Wallis |
	NGT	IGT	T2DM	*P*-value
Age (years)*	58 ± 7	58 ± 7	60 ± 7	0.002
BMI (kg/m²)*	27.5 ± 3.8	29.3 ± 4.2	30.5 ± 4.3	< 0.001
Percent male*	59	61	68	NS
Fasting glucose (mmol/l)*	5.3 ± 0.5	5.9 ± 0.6	7.8 ± 1.8	
Insulin (μU/ml)*	7.2 (5.8–9.9)	9.3 (6.4–13.9)	12.0 (8.4–18.2)	
HOMA-IR*	1.7 (1.3–2.3)	2.3 (1.6–3.7)	4.2 (2.7–6.3)	
ApoB (g/ml)*	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	NS
Cholesterol (mmol/l)	5.3 ± 0.9	5.6 ± 1.0	5.4 ± 1.2	< 0.001
Triglycerides (mmol/l)*	1.2 (0.9–1.7)	1.6 (1.1–2.2)	1.8 (1.2–2.4)	< 0.001
Free fatty acids (mmol/l)*	0.48 ± 0.16	0.55 ± 0.19	0.60 ± 0.21	< 0.001
HDL-cholesterol (mmol/l)*	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	< 0.001
ApoA (g/ml)*	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	0.002
Diastolic BP (mmHg)*	79.1 ± 9.0	82.4 ± 10.3	84.6 ± 9.3	< 0.001
Systolic BP (mmHg)*	128.0 ± 17.0	133.0 ± 18.4	139.8 ± 18.1	< 0.001

*Age, body mass index (BMI), gender and blood pressure and high-density lipoprotein (HDL)-cholesterol were available for 379 normal glucose tolerance (NGT), 228 impaired glucose tolerance (IGT) and 230 Type 2 diabetes mellitus (T2DM) subjects; fasting glucose levels were available for 327 NGT, 157 IGT and 170 T2DM subjects; fasting apolipoprotein (apo) B, triglycerides, free fatty acids, apoA, insulin and homeostasis model assessment of insulin resistance (HOMA-IR) were available for 284 NGT, 128 IGT and 145 T2DM subjects.
Mean ± SD or median (interquartile range).

in 889- and 184-bp fragments for the C-allele and 1073 bp for the T-allele. Restriction fragments were separated on a 1.5% agarose gel and visualized with ethidium bromide. Each time the assay was performed, DNA from a known CC and a known CT individual was included as a positive control (TT DNA was not available).

Statistical analysis

Presence of Gaussian distribution was analysed for all variables. Variables not having a Gaussian distribution were log transformed. Normally distributed data are presented as mean ± SD and differences are assessed by Student's *t*-test/ANOVA; skewed variables are presented as median (interquartile range) and differences are assessed by Mann–Whitney *U*-test/Kruskal–Wallis. No correction for multiple testing was applied since the choice of the variables was largely based on the physiological data available in the TXNIP-deficient mouse model. Using our current knowledge of the allele frequency of the T-allele (see Results), the number of subjects in our analyses provided us with 80% power to detect a statistically significant effect of the *TXNIP* gene when the difference between carriers and non-carriers was 0.3 mmol/l for triglycerides, 0.4 mmol/l for glucose or 5 mmHg for diastolic blood pressure (DBP) (Quanto version 1.1 [23]). For these calculations we used data from a control population representative of Dutch subjects of similar age to our currently used populations, i.e. the spouse control subjects from our study on FCHL [21]. Thirty-two subjects had not stopped their lipid-lowering medication prior to the blood collection and were excluded from the analyses which included lipid parameters. Differences in metabolic parameters were adjusted for age, gender, BMI and site of data sampling, as indicated using linear regression analysis. In linear regression

analyses all standardized residuals were > –3 and < 3, and normal distribution of the residuals was verified. All analyses were done using SPSS 9.0 (SPSS Inc., Chicago, IL, USA).

Results

TXNIP polymorphism and prevalence of diabetes

The allele distributions of the TXNIP C/T polymorphism (rs#2711) were consistent with Hardy–Weinberg equilibrium in all groups. We have analysed this polymorphism in more than 1800 subjects (control, diabetic and hyperlipidaemic subjects from different populations, all White). The mean frequency of carriers of the minor T-allele was 7.8% (range 5.3–13.9%) in different control and patient populations, frequencies which are consistent with data currently reported for populations of White descent (http://www.ensembl.org).

In the population reported here, the frequency of T-allele carriers was not different (χ^2; $P = 0.4$) between groups with different glucose tolerance status, i.e. 7.4% in NGT ($n = 28$ of 379), 10.5% in IGT ($n = 23$ of 219) and 9.3% in T2DM subjects ($n = 20$ of 215). No homozygous carriers of the TXNIP minor allele (TT) were identified.

Effects of the TXNIP genotype in individuals with different glucose tolerance status

Blood pressure
Data on TXNIP genotype and blood pressure were available for 812 subjects (of the 837 included in the study; see also Tables 1

Table 2 Characteristics of carriers and non-carriers of the TXNIP-T allele in NGT, IGT and Type 2 diabetes subjects

| | Non- diabetic subjects | | | | Diabetic subjects | |
| | NGT | | IGT | | T2DM | |
	TXNIP-CC	TXNIP-CT	TXNIP-CC	TXNIP-CT	TXNIP-CC	TXNIP-CT
Cholesterol (mmol/l)†	5.3 ± 0.9	5.3 ± 1.0	5.5 ± 1.0	5.7 ± 0.9	5.4 ± 1.2	5.6 ± 0.8
Triglycerides (mmol/l)†	1.2 (0.9–1.7)	1.3 (1.1–1.5)	1.6 (1.1–2.0)	1.8 (1.4–2.5)	1.6 (1.2–2.3)	2.0 (1.6–5.0)*
Fasting glucose (mmol/l)†	5.3 ± 0.5	5.0 ± 0.5**	5.9 ± 0.7	5.6 ± 0.5	7.8 ± 1.8	7.9 ± 2.2
HOMA-IR†	1.7 (1.4–2.3)	1.7 (1.9–4.8)	2.3 (1.6–3.6)	2.5 (1.9–4.8)	4.3 (2.7–6.4)	3.3 (2.3–5.3)
Diastolic BP (mmHg)†	79.2 ± 9.1	78.6 ± 7.8	82.1 ± 10.7	84.5 ± 10.8	84.0 ± 9.5	89.1 ± 8.6**
Systolic BP (mmHg)†	128.2 ± 17.0	127.5 ± 17.7	132.4 ± 18.4	139.1 ± 19.2	139.1 ± 17.8	143.5 ± 20.9

The data presented are unadjusted mean ± SD or median (interquartile range) values.
†Data on blood pressure were available in 350/28 normal glucose tolerance (NGT), 196/23 impaired glucose tolerance (IGT) and 195/20 Type 2 diabetes mellitus (T2DM) (TXNIP-CC/TXNIP-CT). Fasting glucose was available in 302/22 NGT, 138/16 IGT and 148/12 T2DM (TXNIP-CC/TXNIP-CT). Fasting triglycerides, cholesterol and homeostasis model assessment of insulin resistance (HOMA-IR) were available in 268/16 NGT, 111/15 IGT and 127/9 T2DM (TXNIP-CC/TXNIP-CT). Statistical differences between TXNIP-CC and TXNIP-CT within groups with different glucose tolerance status (NGT, IGT, T2DM): *$P < 0.05$, **$P < 0.025$ (unadjusted P-values); Student's t-test/Mann–Whitney U-test). P-values adjusted for age, gender, sampling site and body mass index (if applicable) are provided in the text.

and 2). DBP was 5.5 mmHg higher in T2DM subjects with the TXNIP-CT genotype than in CC individuals [$P = 0.013$; 95% confidence interval (CI) 1.2, 9.9, adjusted for age, gender and sampling site], but there was no effect in NGT and IGT subjects. Additional adjustment for BMI did not substantially change these data ($P = 0.02$; 95% CI 0.8, 9.5 mmHg). Systolic blood pressure (SBP) did not differ significantly between diabetic carriers of the TXNIP-T allele (143.5 ± 20.9 mmHg) and homozygous diabetic carriers of the major C-allele (139.1 ± 17.8 mmHg; Student's t-test $P = 0.21$, adjusted for age, gender, BMI and sampling site). Urinary albumin concentrations were also higher in diabetic carriers of the TXNIP-T allele [16.1 mg/l (10.2–24.9), median (interquartile range); $n = 7$] than in diabetic TXNIP-CC subjects [6.0 mg/l (3.15–13.05); $n = 101$, $P = 0.019$].

Fasting triglycerides
Data on TXNIP genotype and fasting triglycerides were available for a total of 546 subjects (see Tables 1 and 2). Plasma triglyceride concentrations were 1.6-fold higher in diabetic carriers of the TXNIP minor T-allele than in diabetic CC individuals (95% CI 1.1, 2.3-fold; $P = 0.013$, adjusted for age, gender and sampling site). Additional adjustment for BMI did not substantially change these data ($P = 0.015$; 95% CI 1.1, 2.3). There were no obvious effects of the TXNIP genotype in triglyceride levels in the NGT and IGT subjects.

Indicators of glucose metabolism and insulin resistance
Data on TXNIP genotype and fasting glucose were available for 638 subjects (see Tables 1 and 2). Fasting glucose was 0.26 mmol/l lower in NGT subjects with the TXNIP-CT genotype than in CC individuals ($P = 0.009$; 95% CI −0.45,

−0.063, adjusted for age, gender and sampling site). Fasting glucose levels were also lower in IGT carriers of the TXNIP-CT genotype, but this did not reach statistical significance (0.25 mmol/l; $P = 0.12$, adjusted for age, gender and sampling site). Additional adjustment for BMI decreased P-values slightly ($P = 0.008$ and $P = 0.07$ for NGT and IGT, respectively). Analysis of all non-diabetic subjects combined (i.e. all NGT and IGT subjects), strengthened this relation, resulting in a 0.26 mmol/l lower fasting glucose in non-diabetic carriers of the T-allele compared with non-diabetic TXNIP-CC individuals [$P = 0.002$, 95% CI −0.42, −0.09; adjusted for age, gender, BMI, glucose-tolerance-status (NGT of IGT) and sampling site] This effect of the TXNIP polymorphism on glucose was absent in the T2DM subjects ($P > 0.8$). There were no significant effects of the TXNIP polymorphism on HOMA-IR or plasma insulin concentrations in any of the groups.

Interaction of the TXNIP genotype with parameters of glucose homeostasis
It was recently reported that *TXNIP* is a highly glucose-responsive gene in the pancreatic β-cell [16]. Moreover, Hui *et al.* [17] have suggested that the hyperlipidaemia in the TXNIP-deficient HcB19 mouse model may, at least in part, be secondary to hyperinsulinaemia. We investigated, using linear regression analysis, whether plasma glucose concentrations *per se* or, alternatively, presence of insulin resistance or hyperinsulinaemia, interacted with the *TXNIP* gene polymorphism in the effect on plasma triglyceride levels. There was a significant interaction between fasting plasma glucose concentrations and the *TXNIP* genotype on plasma triglyceride concentration [$P = 0.012$ for the interaction term (TXNIP × glucose) adjusted for age, gender and BMI; Table 3, $n = 544$]. No interaction was observed between plasma insulin

Table 3 Linear regression model describing the interaction between the effects of fasting glucose concentration and the TXNIP polymorphism on plasma triglyceride concentration

	All subjects*				
	B	Std B	*P*-value	R^2	Overall *P*-value
Constant	−0.149		0.10	0.139	< 0.001
Age (years)	−0.002	−0.063	0.12		
Gender (male = 0, female = 1)	−0.020	−0.047	0.24		
Body mass index (kg/m²)	0.008	0.165	< 0.001		
Fasting glucose (mmol/l)†	0.033	0.241	< 0.001		
TXNIP genotype (CC = 0; CT or TT = 1)†	0.072	0.091	0.025		
Interaction (TXNIP genotype × fasting glucose)†	0.049	0.102	0.012		

*Normal glucose tolerance, $n = 283$; impaired glucose tolerance, $n = 126$, Type 2 diabetes mellitus, $n = 135$.
†Centred variables were used to reduce multicollinearity.

concentration or HOMA-IR and the TXNIP polymorphism on plasma triglyceride concentrations [interaction (TXNIP × insulin), $P = 0.9$, interaction (TXNIP × HOMA-IR), $P = 0.3$, adjusted for age, gender, BMI, disease status, TXNIP genotype and insulin/HOMA].

Discussion

Genetic and metabolic data in the current literature prompted us to investigate whether the *TXNIP* gene might play a role in human metabolism, and more specifically in the development of human diabetes and/or the hyperlipidaemia associated with diabetes. *TXNIP* is a highly glucose-responsive gene [16] and a recent report by Minn *et al.* [24] has shown that glucose directly stimulates TXNIP expression via a carbohydrate response element (CHOre) in the promoter of this gene. The overall frequency of the TXNIP variation did not differ between diabetic and non-diabetic subjects, implying that this polymorphism did not affect the risk of T2DM in these subjects. However, genetic variations in the *TXNIP* gene did significantly affect several parameters associated with diabetes.

The TXNIP polymorphism influences triglyceride metabolism. Plasma triglyceride concentrations were elevated in diabetic carriers of the TXNIP-T allele, but not in non-diabetic T carriers. This was confirmed in additional analyses: triglyceride concentrations were influenced by a significant interaction between plasma glucose concentration and the TXNIP polymorphism. This latter aspect is of particular interest because of the previously mentioned regulation of TXNIP expression by glucose [16] and suggests that the biological relevance of TXNIP variations may be particularly important when glycaemic control is inadequate. High plasma glucose concentrations in diabetes reflect insufficient amounts of plasma insulin, which is most likely associated with a relative glucose deficit in the cells and might subsequently compromise TXNIP expression. This effect might be more pronounced, i.e. may have greater metabolic consequences, in carriers of the T-allele. Indeed,

hyperglycaemia in diabetes is often associated with hypertriglyceridaemia [25,26]. Interestingly, interaction between TXNIP and plasma glucose concentration on plasma triglyceride could also explain, at least in part, the absence of effect of the TXNIP polymorphisms on hypertriglyceridaemia in FCHL [8–10] that was confirmed in our own FCHL patients (data not shown), because FCHL patients generally remain normo-glycaemic, despite insulin resistance and hyperinsulinaemia.

Our data suggest that the potential of an individual to utilize glucose for energy homeostasis, which is altered in diabetes, may interfere with a metabolic effect of the TXNIP polymorphism. The TXNIP-T allele is associated with lower fasting glucose levels in the non-diabetic state, but with hypertriglyceridaemia in diabetic subjects. This is in line with previously reported mouse data. It has been suggested that TXNIP may act as a regulator or switch in fuel utilization, possibly via changes in the cellular redox status [17,27]. Absence of TXNIP in mice induced, among others, an altered hepatic response to nutrient signals [17] and disturbances in the fasting–refeeding transition [27] and, of interest, both these studies reported reduced fasting glucose concentrations in TXNIP-deficient mice.

Our present data are based on the results of one SNP in the 3′ UTR of the *TXNIP* gene, and not all metabolic variables were available for all subjects. In addition, we used a relatively small study population, although the power calculation suggested that we did have acceptable power to detect the differences reported here. A larger study population would possibly also have allowed us to detect more subtle differences. Despite these limitations, it is noteworthy that two of the three variables found to be affected by this TXNIP polymorphism in our human study population, i.e. triglycerides and glucose, match those that were earlier reported in the murine model [4,7,17,27,28]. The phenotypic changes associated with the minor TXNIP T-allele in our human subjects were analogous to those reported in the TXNIP-deficient HcB19 mouse model. This suggests that the most likely effect of the TXNIP T-allele is a decreased amount of (functional) TXNIP protein, possibly

accompanied by higher cellular thioredoxin activity. A possible consequence of a polymorphism in the 3′ UTR region of a gene is a change in RNA stability or translation efficiency. The position of rs#7211 is in the proximal one-third of the 3′ UTR, almost 1000 bases away from the poly A tail. Moreover, the region surrounding the SNP is not particularly AU-rich. It is therefore unlikely to influence the stability through influencing the poly A tail. Analysis the human and rat 3′ UTR sequence with rVista 2.0/Transfac [29] revealed a conserved binding site covering the SNP for germ cell nuclear factor (GCNF). However, based on the currently known functions of GCNF, which are mainly related to embryonic development and germ cell maturation (as reviewed in [30]), this factor does not appear to be a very plausible candidate for regulation of TXNIP. Therefore, the exact implications of the presence of this particular SNP remain to be established.

The TXNIP-T allele was also associated with higher levels of DBP in the diabetic subjects. Blood pressure had not been previously addressed in the murine model and we had no clear prior expectations of effects of this polymorphism on blood pressure. The association between higher DBP and the presence of the minor TXNIP T-allele was independent of plasma triglyceride concentrations and BMI. As discussed above, the TXNIP-T allele is most likely associated with decreased TXNIP activity and, consequently, with higher cellular thioredoxin activity. An interesting aspect in this regard is that thioredoxin can induce the expression of vascular endothelial growth factor (VEGF) [31]. This reveals a possible pathway via which the TXNIP-T allele may be associated with higher VEGF expression. Increased VEGF concentrations, in turn, have been reported to induce diabetic nephropathy [32,33], which is reflected by, for example, microalbuminuria. It is well known that microalbuminuria is associated with increased blood pressure [34,35]. Thus, the presence of the TXNIP-T allele, if associated with lower cellular levels of TXNIP and increased thioredoxin activity, higher VEGF expression and increased microalbuminuria, could potentially explain, at least in part, the increase in DBP observed in the diabetic carriers of the TXNIP-T allele. Although this mechanism is highly speculative, the higher DBP in the diabetic carriers of the TXNIP-T allele we report in our current study was indeed accompanied by significantly increased urinary albumin concentrations, suggesting that renal dysfunction was commoner in these subjects.

In conclusion, we have established that interaction between a genetic variation in the *TXNIP* gene and fasting plasma glucose affects plasma triglyceride concentrations in human subjects. This suggests that variations in this gene may be particularly relevant in human metabolic disorders with recurrent episodes of poor glycaemic control. Indeed, the 3′ UTR polymorphism in TXNIP was specifically associated with hypertriglyceridaemia in human Type 2 diabetic subjects. In addition, an effect on DBP was seen which was independent of plasma triglyceride concentrations. The association of the TXNIP polymorphism with hypertriglyceridaemia and increased blood pressure may potentially contribute to cardiovascular risk in T2DM.

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