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Thioredoxin interacting protein genetic variation is associated with diabetes and hypertension in the Brazilian general population

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ABSTRACT

Objective: To investigate the relationship between *TXNIP* polymorphisms, diabetes and hypertension phenotypes in the Brazilian general population.
Methods: Five hundred seventy-six individuals randomly selected from the general urban population according to the MONICA-WHO project guidelines were phenotyped for cardiovascular risk factors. A second, independent, sample composed of 487 family-trios from a different site was also selected. Nine *TXNIP* polymorphisms were studied. The potential association between *TXNIP* variability and glucose-phenotypes in children was also explored. TXNIP expression was quantified by real-time PCR in 53 samples from human smooth muscle cells primary culture.
Results: TXNIP rs7211 and rs7212 polymorphisms were significantly associated with glucose and blood pressure related phenotypes. In multivariate logistic regression models the studied markers remained associated with diabetes even after adjustment for covariates. *TXNIP* rs7211 T/rs7212 G haplotype (present in approximately 17% of individuals) was significantly associated to diabetes in both samples. In children, the *TXNIP* rs7211 T/rs7212 G haplotype was associated with fasting insulin concentrations. Finally, cells harboring *TXNIP* rs7212 G allele presented higher TXNIP expression levels compared with carriers of *TXNIP* rs7212 CC genotype ($p = 0.02$).
Conclusion: Carriers of *TXNIP* genetic variants presented higher TXNIP expression, early signs of glucose homeostasis derangement and increased susceptibility to chronic metabolic conditions such as diabetes and hypertension. Our data suggest that genetic variation in the *TXNIP* gene may act as a "common ground" modulator of both traits: diabetes and hypertension.

1. Background

Diabetes and hypertension are growing global health problems. Both conditions have a complex etiology involving the interaction between genetic and environment factors, such as diet, obesity, oxidative stress, and sedentarism [1,2]. Diabetes incidence increases rapidly worldwide probably due to the explosive increase in obesity over the last decade [2]. Individuals with previously undiagnosed diabetes have an unfavorable cardiovascular risk profile compared with glucose tolerant individuals [3–5]. Several studies have been performed with the aim of identifying candidate loci in pathways implicated both in glucose and blood pressure homeostasis [4,6–8].

The TXNIP protein (thioredoxin interacting protein) was first isolated from a 1,25-dihydroxyvitamin D3-treated HL-60 human promyelocytic cell line and therefore called vitamin D3-up-regulated gene 1 (*vdup*) [9]. This protein inhibits the thioredoxin (TXN) system functioning as an endogenous inhibitor [10]. TXN is a multifunctional antioxidant enzyme expressed in various tissues [11] and plays an indirect, but essential, role in signaling molecules by protein–protein interactions [12].

To date, *TXNIP* gene has been identified as one of the most dramatically up-regulated genes in studies looking at the genomic effect of glucose on isolated islet cells [13]. TXNIP is over-expressed

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in diabetes and has deleterious effects on pancreatic β cells. In addition, TXNIP affected INS-1 β cell gene expression and TXNIP over-expression impairs glucose induced insulin secretion and it has been hypothesized that the *TXNIP* gene represents a link between glucose-toxicity and β cell apoptosis, two critical mechanisms in the pathogenesis of diabetes [14]. In addition, it appears that *TXNIP* is also modulated by mechanical stimuli from the cardiovascular system, as occurs during pressure-overload cardiac hypertrophy or even physiologic fluid shear stress [15].

Here, the main aim was to investigate the relationship between *TXNIP* genetic variation and diabetes and hypertension phenotypes in a large sample from an ethnically mixed Brazilian population. In addition, through replication studies and *ex vivo* gene expression experiments we aimed at identifying the potential molecular link between *TXNIP* polymorphisms and diabetes and hypertension.

2. Methods

2.1. Study samples

2.1.1. Derivation sample

A cross-sectional study of risk factors for cardiovascular diseases was performed in the urban population of Vitoria, Espirito Santo State, Brazil, using the WHO-MONICA project guidelines [16]. A sample of 2044 individuals (from an eligible population of 137,330) of either gender, 25–64 years old, was chosen according to the nearest birthday after a random selection of domiciles. Ethnicity was categorized as Caucasian-descents, African-descents and racially mixed individuals [17]. In present analysis, data from 576 participants were used. Physical examination emphasized measurement of height, weight, smoking habits, and alcohol consumption.

Blood pressure was measured using a standard mercury sphygmomanometer on the left arm after 5 min rest, in the sitting position. Systolic (SBP) and diastolic (DBP) blood pressures were calculated from three readings with a minimal interval of 5 min. Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and/or use of anti-hypertension drugs [18].

Blood glucose, total cholesterol, lipoprotein fractions, and triglycerides were assayed by standard techniques in 12-h fasting blood sample. Diabetes mellitus was defined as a fasting glucose ≥ 126 mg/dL and/or use of hypoglycemic drugs. No participant reported presence of type 1 diabetes mellitus. Body mass index (BMI) (weight in kg/height in meters [2]) was calculated. Height was measured in centimeters and weight in kilograms using a calibrated balance. Supine waist girth was measured at the umbilicus level [19].

2.1.2. Replication sample

The Itapetininga study population comprised 487 family-trios. Each trio was composed of 1 child and at least 1 parent (total 865 parents). Children, 2–18 years old, were randomly selected from the 89 public schools in the Itapetininga City, Sao Paulo State, Brazil, after informed consent was obtained from the parents. Ethnicity (self-referred) information for this sample was used in the present analysis for model adjustment similarly as in the derivation sample. Cardiovascular risk factors were evaluated by a standard questionnaire directed to both the child and the parents along with a detailed medical examination. Blood pressure was measured using appropriate cuffs for arm length on mercury sphygmomanometers with the subject in a sitting position after a 5-min rest. Blood samples were collected after a 12-h fast. Total cholesterol, lipoprotein fractions, triglycerides, and blood glucose were determined by standard enzymatic methods. High sensitivity assay for C-reactive protein was performed by immunochemistry (Cardiophase®, Dade Behring, MA, USA). Insulin was determined by electrochemiluminescence (Roche Diagnostics, Mannheim, Germany).

In the present analysis, data from the 865 parents were used as a replication sample. Data from the children plus parents in a family-based association study design were used for dissecting potential early effects of the observed risk genotypes on glucose homeostasis on children.

2.2. Assessment of genotypes for the TXNIP gene polymorphism

Nine *TXNIP* polymorphisms (rs833392, rs2791749, rs2791750, rs6674773, rs7548422, rs7211, rs7212, rs4755, and rs13203) were selected as representative of the genetic variation at this locus using information available from the HapMap Project homepage (www.hapmap.org) and the dbSNP database (www.ncbi.nlm.nih.gov). However, only *TXNIP* rs833392, rs7211, rs7212, and rs4755 were significantly polymorphic in our sample to be used in the analysis.

Genomic DNA was extracted from peripheral blood leukocytes by means of a salting-out procedure. Table 1 in the Supplementary appendix shows primers and restriction endonucleases used in the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism assay). A 30-cycle PCR was carried out using a 10 μL reactive solution containing (10 mM Tris–HCl, pH 9.0; 50 mM KCl; 2.5 mM $MgCl_2$; 100 μM of each dNTP; 0.3 U *Taq* DNA polymerase; 5 pmol of each primer; 50 ng of genomic DNA template). PCR products were digested with 1 U of the appropriate restriction enzyme and visualized by 3% agarose gel electrophoresis. Quality control for these assays was assessed by randomly selecting 40 samples for re-genotyping. One-hundred percent concordance was observed in these tests.

2.3. Vessel harvesting and primary culture of smooth muscle cells (SMC)

Human mammary artery (h-MA) segments were obtained from patients undergoing aortocoronary bypass surgery at the Heart Institute (InCor), University of Sao Paulo Medical School. All individuals gave informed consent to participate in the study, which was reviewed and approved by the local Ethics Committee (SDC 2454/04/074-CAPPesq 638/04). Cells were obtained by explant protocol and cultured with Dulbecco's modified Eagle's medium containing 20% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin. Primary culture cells were expanded until the third passage, when total RNA was extracted for gene expression quantification. Smooth muscle cells were characterized by hill-and-valley growth pattern and by immunofluorescence staining for α-smooth muscle actin [20].

2.4. Gene expression by real Time reverse transcription polymerase chain reaction (RT-PCR)

Quantitative RT-PCR was performed in h-MA primary culture cells. Total RNA was isolated with Trizol LS Reagent® according to the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA, USA) and cDNA synthesis was performed with random hexamers (High Capacity cDNA Archive®; kit-PE Applied Biosystems, Foster City, CA, USA). Five nanograms of cDNA were used for real-time reaction by *SYBR® Green PCR Master Mix-PE – ABI Prism 7500 Fast System®* (Applied Biosystem, Foster City, CA, USA). All samples were assayed in triplicate and 28S ribosomal and *cyclophilin* gene RNA were used as an internal control to normalize the results. Efficiencies of TXNIP and 28S and cyclophilin amplification reactions were very similar and close to 1. Comparative threshold (C_T) cycle

Table 1
Demographic and biochemical data of the studied populations.

	Derivation sample Vitoria	Replication sample Itapetininga	*p* Value
Total sample	576	865	
Gender (female), %	59.9	52.5	0.005
Diabetes, %	9.0	3.6	<0.001
Hypertension, %	37.0	10.6	<0.001
Age, years	44.5 ± 11.0	39.8 ± 8.0	<0.001
Glucose, mg/dL	105.5 ± 32.8	91.5 ± 29.4	<0.001
Triglycerides, mg/dL	133.0 ± 94.3	146.0 ± 102.5	<0.001
Total cholesterol, mg/dL	219.0 ± 44.8	197.4 ± 42.2	<0.001
HDL cholesterol, mg/dL	45.0 ± 11.6	44.4 ± 12.2	0.193
LDL chelesterol, mg/dL	148.0 ± 40.9	124.1 ± 37.0	<0.001

HDL: high-density lipoprotein; LDL: low-density lipoprotein.

method was used for data analyses. ΔC_T is the difference in threshold cycle for target (TXNIP) and reference (28S and *cyclophilin*).

2.5. Statistical analysis

Kolmogorov–Smirnov and Shapiro–Wilk tests were used for assessing sample distribution. Continuous variables were compared using one-way ANOVA (analysis of variance) and presented as mean ± SD (standard deviation). Categorical variables were compared using Chi-square test and presented as percentage. Multivariate linear regression models were created for adjustment for potential confounding variables. Logistic regression analysis was carried out to estimate the odds ratio (OR), in order to assess genetic risk factors for diabetes and hypertension according to *TXNIP* polymorphisms. Biochemical data, blood pressures, diabetes and hypertension phenotypes were adjusted for age, gender and BMI. In the Vitoria sample ethnicity was available and logistic regression models were also adjusted for this confounder. Ethnicity was entered into the model as dummy variables (0/1 for Caucasian ethnicity and 0/1 for Mulatto ethnicity).

Gene expression was presented as mean $2^{-\Delta C_T}$ plus standard deviations and differences among *TXNIP* groups were analyzed using the Students *t*-test by comparing differences in the mean levels of each genotype. Statistical analyses were carried out using the SPSS 16.0 software, with the level of significance set at $p < 0.05$.

Hardy–Weinberg equilibrium, linkage disequilibrium (LD), and haplotypic association analysis were conducted with Haploview 4.0 software [21]. Quantitative transmission disequilibrium test (QTDT) software package was used for linkage and LD studies on the family-trios structure data. Analysis using allele and haplotype data for continuous traits were conducted using the HAPSTAT Program.

3. Results

3.1. Demographic and genetic structure data

Demographic data of the studied population samples are shown in Table 1. Allelic, genotypic and haplotypic data are presented in Table 2 in the Supplementary appendix.

All studied *TXNIP* polymorphisms were in accordance with the Hardy–Weinberg equilibrium. Pairwise linkage disequilibrium (LD) analysis has shown significant disequilibrium between the *TXNIP* rs833392, rs7211, and rs7212 (Fig. 1 in the Supplementary appendix). *TXNIP* rs4755 had no strong disequilibrium with other markers assayed in the *TXNIP* gene (Fig. 1 in the Supplementary appendix).

Table 2
Relationship between *TXNIP* polymorphisms and glucose and blood pressure.

Alleles	Glucose (mg/dL)	SBP (mmHg)	DBP (mmHg)
Derivation sample Vitoria			
rs7211 C	108.6 ± 2.1	129.0 ± 1.4	84.3 ± 0.8
rs7211 T	114.6 ± 3.6	132.1 ± 2.3	85.9 ± 1.4
p values	0.005/0.05	0.03/0.34	0.05/0.45
rs7212 C	107.5 ± 2.4	129.6 ± 1.6	84.8 ± 0.9
rs7212 G	110.7 ± 4.2	133.1 ± 2.7	86.9 ± 1.6
p values	0.18/0.63	0.03/0.29	0.02/0.25
rs4755 A	105.8 ± 2.4	129.8 ± 1.5	85.3 ± 0.9
rs4755 T	106.5 ± 4.0	134.1 ± 2.6	87.4 ± 1.5
p values	0.77/0.68	0.05/0.03	0.005/0.04
Replication sample Itapetininga			
rs7211 C	90.7 ± 1.8	122.1 ± 1.5	78.3 ± 1.0
rs7211 T	91.2 ± 2.9	124.0 ± 2.4	79.6 ± 1.6
p values	0.78/0.90	0.14/0.37	0.17/0.39
rs7212 C	92.3 ± 1.9	122.0 ± 1.5	78.3 ± 1.0
rs7212 G	93.5 ± 3.3	123.9 ± 2.7	79.5 ± 1.8
p values	0.51/0.40	0.21/0.33	0.24/0.42
rs4755 A	91.4 ± 1.9	123.5 ± 2.8	78.3 ± 1.2
rs4755 T	91.8 ± 3.5	126.0 ± 3.2	78.9 ± 1.3
p values	0.78/0.13	0.08/0.35	0.75/0.75

Number of alleles for the rs7211 (887 *vs* 265 and 1384 *vs* 346), rs7212 (946 *vs* 206 and 1453 *vs* 277), rs4755 (899 *vs* 253 and 1073 *vs* 657), in the Vitoria and Itapetininga samples, respectively. These analysis were performed for BMI, total cholesterol and triglycerides, but no significant difference was observed according to *TXNIP* alleles. *TXNIP* rs833392 alleles did not demonstrate association with the analyzed variables. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. Data shown as mean ± standard error for univariate (unadjusted analysis). Provided *p* values are for unadjusted and adjusted (age, gender, BMI and ethnicity) analysis, respectively.

In further analysis, the two polymorphisms with higher LD (rs7211 and rs7212) were used for haplotype-based analysis. All tested polymorphisms were also analyzed as one block, but results based on haplotypes defined by SNPs rs7211 and rs7212 presented best fit compared to association analyzes using haplotypes defined by all 4 polymorphisms. In addition, low haplotypic diversity was observed in this locus. Only 3 different haplotypes could explain more than 90% of the overall genetic diversity of this locus in the studied sample (Table 2 in the Supplementary appendix).

3.2. Relationship between TXNIP alleles and blood glucose and blood pressure

Comparisons for each genetic variant were performed using *p*-values unadjusted and adjusted for age, gender, BMI, and ethnicity (Table 2). In the Vitoria sample, after univariate analysis, association was disclosed between *TXNIP* rs7211 and blood glucose concentrations ($p = 0.005$) and both SBP and DBP ($p = 0.03$); and for *TXNIP* rs7212 and *TXNIP* rs4755 for SBP ($p = 0.03$ and $p = 0.03$) and DBP ($p = 0.05$ and $p = 0.02$), respectively (Table 2, *p*-values for unadjusted analysis).

In the Vitoria sample, *TXNIP* rs7211 T allele persisted significantly associated with glucose concentrations after adjustment for age, gender, BMI, and ethnicity ($p = 0.052$).

In the replication sample, the *TXNIP* rs7211 did not present association with glucose concentrations, SBP and DBP (Table 2).

No relationship between *TXNIP* polymorphism alleles and total cholesterol, lipoprotein fractions, triglycerides and BMI was found (data not shown).

3.3. Relationship between TXNIP alleles, diabetes and hypertension phenotypes

TXNIP rs7212 G allele frequencies in diabetic and non-diabetic individuals were significantly different and replicated in both

Table 3
Relationship between *TXNIP* haplotypes and glucose and blood pressure.

Haplotypes Derivation sample Vitoria	Glucose (mg/dL) $n=576$	SBP (mmHg) $n=576$	DBP (mmHg) $n=576$
rs7211 C/rs7212 C	109.8 ± 5.3 (*vs* 114.1)	128.7 ± 3.8	84.2 ± 2.2
p values	0.007/0.06	0.04/0.41	0.06/0.46
rs7211 T/rs7212 C	115.9 ± 5.1 (*vs* 104.1)	127.7 ± 3.7	83.1 ± 2.2
p values	0.005/0.009	0.88/0.79	0.79/0.57
rs7211 T/rs7212 G	107.7 ± 4.1	129.7 ± 2.7	84.7 ± 1.6
p values	0.14/0.55	0.02/0.23	0.02/0.24
Haplotypes Replication sample Itapetininga	**Glucose (mg/dL) $n=804$**	**SBP (mmHg) $n=498$**	**DBP (mmHg) $n=500$**
rs7211 C/rs7212 C	91.2 ± 4.6	121.8 ± 3.8	78.2 ± 2.8
p Values	0.74/0.86	0.15/0.37	0.18/0.35
rs7211 T/rs7212 C	87.3 ± 4.5	121.6 ± 4.2	78.6 ± 2.8
p Values	0.06/0.06	0.64/0.91	0.65/0.92
rs7211 T/rs7212 G	92.4 ± 4.4	122.9 ± 2.8	78.1 ± 1.9
p Values	0.47/0.37	0.20/0.38	0.23/0.33

These analysis were performed for BMI, total cholesterol and triglycerides, but no significant difference was observed according to *TXNIP* haplotypes. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure. Data shown as mean ± standard error for univariate (unadjusted analysis). Provided *p* values are for unadjusted and adjusted (age, gender, BMI and ethnicity) analysis, respectively. Lower case values represent estimated unadjusted mean values for all other haplotypes.

samples in models adjusted for age, gender, BMI, and ethnicity (*p* value for unadjusted and adjusted analysis in derivation sample = 0.001 and 0.04, respectively; *p* value for unadjusted and adjusted analysis in replication sample = 0.04 and 0.04, respectively). Higher diabetes odds in both population samples were associated with rs7212 G allele (OR, 2.50; 95% CI, 1.30–4.60 and OR, 1.85; 95% CI, 1.02–3.33) ($p=0.006$ and $p=0.025$, respectively for the derivation and replication samples).

The *TXNIP* rs7211 T allele was also associated with hypertension (OR, 2.26; $p=0.005$ and OR, 2.42; $p=0.001$) for both Vitoria and Itapetininga samples, even after adjustment for covariates.

In the Vitoria sample, presence of at least one T allele of the rs7211 marker was associated with diabetes (OR, 2.78; 95% CI, 1.40–5.50). Likewise, presence of at least one G allele of the rs7212 marker was also associated with diabetes (OR, 2.36; 95% CI, 1.22–4.59). For hypertension, both markers were not significant anymore after the adjustment for ethnicity.

3.4. Relationship between TXNIP haplotypes and blood glucose and blood pressure

LD for all possible two-way comparison among the 4 selected *TXNIP* polymorphisms was investigated.

In the Vitoria sample, lower and higher mean values of blood glucose were observed in the *TXNIP* rs7211 C/rs7212 C and rs7211 T/rs7212 C haplotype groups ($p=0.005$ and $p=0.009$, respectively), compared with other haplotype groups (Table 3). This was true even after adjustment for age, gender, BMI, and ethnicity (Table 3). This was not replicated in the replication sample (Table 3).

No relationship between *TXNIP* haplotypes and total cholesterol, lipoprotein fractions, triglycerides and BMI was found.

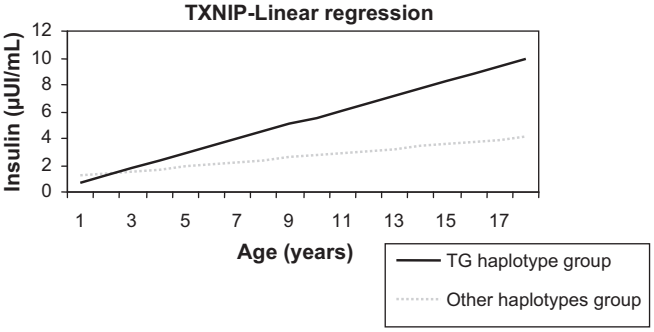

Fig. 1. Prediction curves of insulin level taken into consideration haplotype status and participant age.

3.5. Relationship between TXNIP haplotypes and diabetes and hypertension phenotypes

In an unadjusted model, the presence of *TXNIP* rs7211 T/rs7212 G haplotype was associated with diabetes in both Vitoria and Itapetininga samples, respectively (Table 4).

Haplotype analysis adjusting for age, gender, BMI, and ethnicity were concordant and showed a significantly higher frequency of diabetes in individuals harboring the rs7211 T/rs7212 G haplotype in both Vitoria ($p=0.05$) and Itapetininga ($p=0.03$) samples.

3.6. TXNIP genetic variants and glucose homeostasis in children

Haplotype analyses for several variables associated with glucose homeostasis were conducted (Table 2, Supplementary appendix). Children carrying *TXNIP* rs7211 T/rs7212 G haplotype were associated with higher blood insulin values (3.85 µUI/mL) compared with other haplotype groups (2.76 µUI/mL), even after adjustment for age and age *genotype interaction ($p=0.032$). Of particular importance is the fact that one should analyze these results taken into account the fact that the interaction term of this model was also statistically significant. This makes it difficult to interpret the sole genotype effect since it depends on the participant age (Fig. 1). Nonetheless, this analysis suggests that for the identified risk haplotype carriers increased insulin levels are predicted as one grows older. This information is important in our formulating model to explain the effect of TXNIP genetic variability and diabetes (see Fig. 1 and Section 4).

A computer-model was performed to predict the homeostatic concentrations which arise from varying degrees of β-cell deficiency and insulin resistance (HOMA – homeostasis model assessment) [22]. The effect of *TXNIP* rs7211 T/rs7212 G haplotype on children was not observed for these phenotypes (Table 2, Supplementary appendix).

3.7. TXNIP gene expression

An RT-PCR assay was developed to characterize TXNIP mRNA expression in smooth muscle cells. Cells from 53 individuals were used. From these, 42 had the rs7212 CC genotype, and 11 had the rs7212 CG genotype.

Cells carrying *TXNIP* rs7212 CG had higher TXNIP expression (mean $2^{-\Delta C_T}$, 0.18; s.d., 0.10) compared with carriers *TXNIP* rs7212 CC (mean $2^{-\Delta C_T}$, 0.10; s.d., 0.08) ($p=0.01$). No association between TXNIP gene expression and rs7211 genotypes was observed, but a tendency toward increased expression levels in individuals harboring the T allele was also observed (CC ($n=43$) mean $2^{-\Delta C_T}$, 0.10; s.d., 0.08 and CT ($n=10$) mean $2^{-\Delta C_T}$, 0.14; s.d., 0.09).

Table 4
Analysis of diabetes and hypertension association with case-control *TXNIP* haplotype frequencies.

	Diabetes				Hypertension			
	Case	Control	*p* value	Adjusted *p* value	Case	Control	*p* value	Adjusted *p* value
Derivation sample								
rs7211 C/rs7212 C	0.62	0.78			0.74	0.78		
rs7211 T/rs7212 C	0.00	0.00	0.006	0.05	0.00	0.00	0.13	0.65
rs7211 C/rs7212G	0.09	0.05			0.06	0.05		
rs7211 T/rs7212G	0.28	0.17			0.20	0.16		
Replication sample								
rs7211 C/rs7212 C	0.72	0.78			0.71	0.79		
rs7211 T/rs7212 C	0.00	0.00	0.04	0.03	0.00	0.00	0.02	0.10
rs7211 C/rs7212G	0.00	0.05			0.05	0.04		
rs7211 T/rs7212G	0.28	0.17			0.24	0.16		

Adjusted analysis for age, gender, body mass index, ethnicity (Caucasian yes/no and Mulatto yes/no). Number of individuals in each subgroup: diabetic cases derivation sample: 52, number of diabetic controls derivation sample: 524. Diabetic cases replication sample: 29, number of diabetic controls replication sample: 775. Hypertension cases derivation sample: 203, hypertension controls derivation sample: 373. Hypertension cases replication sample: 94, hypertension controls derivation sample: 406.

4. Discussion

Significant relationship between *TXNIP* genetic variation and diabetes and hypertension were observed in two general samples from the general population, Itapetininga and Vitoria. In addition, blood glucose, SBP and DBP, as continuous variables, were associated to *TXNIP* markers. Interestingly, multivariate logistic regression models were able to demonstrate the association of the studied markers with diabetes, even after adjustment for their known covariates (age, gender and BMI) plus ethnicity. Finally, we were also able to observe derangements in glucose homeostasis associated with the identified risk haplotype in children.

TXNIP protein is an endogenous inhibitor of thioredoxin, and plays a pivotal role in several important processes of cardiovascular homeostasis by functioning as a biological sensor for biomechanical and oxidative stress [12]. It appears that *TXNIP* is also modulated by mechanical stimuli from the cardiovascular system, as occurs during pressure-overload cardiac hypertrophy or even physiologic fluid shear stress [15].

Few studies to date have aimed at investigating the possible role of *TXNIP* genetic variation on cardiovascular phenotypes. Coon et al., using a subset of families from the NHLBI Family Heart Study, researched the impact of *TXNIP* genetic variation on lipid and serum glucose concentrations, but they did not find any association with the studied phenotypes [23]. Nevertheless, they did not study rs7211 and rs7212, our two associated markers. It is possible that the previously used markers were not able of capturing a significant amount of this locus overall variability, limiting the statistical power of their approach. van Greevenbroek et al. using metabolic data investigated whether *TXNIP* polymorphisms determine an effect on parameters associated with lipid and glucose metabolism in humans [24]. Plasma triglycerides concentrations were higher in diabetic individuals carrying *TXNIP* rs7211 T allele than diabetics with the common C allele [24]. The authors concluded that effect of TXNIP on triglycerides is influenced by plasma glucose concentrations, suggesting that the biological relevance of *TXNIP* variation may be particularly relevant in recurrent episodes of hyperglycemia [24]. Our study did not find any association with triglycerides in diabetic individuals, but one should note that our reduced number of diabetic individuals might have reduced our sensitivity to detect such association.

Parikh et al. combined genome-wide expression profiling, genetic association testing and cellular studies to spotlight TXNIP protein as a physiologic regulator of peripheral glucose uptake in humans [25]. Despite studying a sample of 4450 Scandinavian individuals, they did not found any significant association between *TXNIP* tag SNPs, and diabetes or quantitative phenotypes, as fasting glucose, insulin and HOMA β [25]. Nonetheless neither rs7211 nor rs7212 were part of their tag SNP list.

The *TXNIP* rs7211 T/rs7212 G haplotype (present in approximately 17% of Brazilian individuals) has consistently shown to be associated with diabetes in both population samples: Vitoria and Itapetininga (OR, 1.94; 95% CI, 1.23–3.07 and OR, 1.89; 95% CI, 1.04–3.41, respectively). TXNIP was over-expressed in h-MA of the individuals with *TXNIP* rs7212 CG group compared with CC group. Finally, in children, insulin concentrations were higher in carriers of *TXNIP* rs7211 T/rs7212 G haplotype. This dependence was mainly observed in older children, suggesting that the age *genotype relationship may be increasing the risk of glucose derangements as the individual gets older.

A *TXNIP*-diabetes pathogenic hypothesis suggested by our group, based on published data, was that *TXNIP* regulates both insulin-dependent and insulin-independent pathways of glucose uptake [26]. TXNIP protein is an endogenous inhibitor of thioredoxin enzyme expressed in vessels, which is a gateway of cell damage. Thioredoxin is a key component of redox regulation and has been indicated to play an essential role in cell survival and growth [27]. TXNIP expression was inversely correlated with insulin-stimulated glucose uptake and that TXNIP expression was elevated by glucose in human muscle cells [25]. It is known that hyperglycemia induced oxidative stress modulated by TXNIP induction, with the consequent inhibition of the anti-oxidative function [28]. In addition, *TXNIP* is dramatically induced by glucose [27] and, in hyperglycemia, inhibits thioredoxin with accompanying impairment in vascular network formation [27,29].

Taken together, one could formulate a model in which *TXNIP* genetic variation may modulate both diabetes and vascular risk as follows: TXNIP increased expression (associated with the *TXNIP* rs7211 T/rs7212 G haplotype) decreases glucose uptake in muscle cells and thereby increases the concentrations of circulating insulin and glucose, explaining the data observed in both children and adults. The increased glucose in the pancreas leads to glucose-toxicity causing a progressive reduction of β cell secretion. The net result is hyperglycemia. In the hyperglycemic status, individuals with *TXNIP* rs7211 T/rs7212 G haplotypes are more susceptible to have altered gene expression in the pancreas resulting in an even greater loss of the mass of β cells by apoptosis induced by the TXNIP protein.

Our study has some limitations. First, there were no replications for the differences in glucose and blood pressures as continuous variables. A possible explanation can be the significant different mean age between the Vitoria and Itapetininga population samples. Nonetheless, association between diabetes and hypertension according to *TXNIP* polymorphisms was replicated in both population samples, even with a lower frequency of diabetes and hypertension cases in the Itapetininga subjects. Second, despite the positive results and the replication of the results for dichotomous traits, especially diabetes, we have worked with a small number

of diabetic individuals. In order to reduce the concern of false positive results one should take into consideration that several lines of evidence point toward the conclusion of the observed association: replication in both samples, replication with an intermediate phenotype in children and evidence of the association with gene expression levels in *ex vivo* experiments. Finally, we were not able to specifically determine the molecular alteration responsible for the observed association. It is tempting, however, to suggest that genetic variation in the 3′ portion of TXNIP RNA is responsible for the differential gene expression observed. In fact, both rs7211 and rs7212 are located in the 3′ region and may be susceptible to different gene expression regulating mechanisms (*i.e.*, modulate RNA stability, microRNA binding, etc.).

Nonetheless, in the described scenario, our data plus literature data provide evidence that the *TXNIP* gene may constitute not only a gateway for biomechanical transduction in the cardiovascular system, but may also be a fundamental link between both epidemiological and mechanistic studies involving diabetes and hypertension.

To the best of our knowledge, this was the first study analyzing *TXNIP* polymorphisms and diabetes and hypertension phenotypes in both derivation and replication population samples. These genetic associations, although exploratory regarding casual relations, may have a role in the generation of hypothesis to be tested in more controlled studies.

In conclusion, individuals harboring specific *TXNIP* genetic constitution present higher levels of TXNIP expression, early signs of glucose homeostasis derangement and increased susceptibility to chronic metabolic conditions such as diabetes and hypertension.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.12.009.

References

[1] Rich SS. Genetics of diabetes and its complications. J Am Soc Nephrol 2006;17:353–60.
[2] Zidek W, Naditch-Brule L, Perlini S, Farsang C, Kjeldsen SE. Blood pressure control and components of the metabolic syndrome: the good survey. Cardiovasc Diabetol 2009;8:51.
[3] Mokdad AH, Ford ES, Bowman BA, et al. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 2003;289:76–9.
[4] De Flines, J. and Scheen, A.J., Management of metabolic syndrome and associated cardiovascular risk factors, Acta Gastroenterol Belg, 73: 261–266.
[5] Glumer C, Jorgensen T, Borch-Johnsen K. Prevalences of diabetes and impaired glucose regulation in a Danish population: the Inter99 study. Diabetes Care 2003;26:2335–40.
[6] Redon J, Oliva MR, Tormos C, et al. Antioxidant activities and oxidative stress byproducts in human hypertension. Hypertension 2003;41:1096–101.
[7] Yasunari K, Maeda K, Nakamura M, Yoshikawa J. Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein. Hypertension 2002;39:777–80.
[8] Harrison DG. The shear stress of keeping arteries clear. Nat Med 2005;11:375–6.
[9] Chen KS, DeLuca HF. Isolation and characterization of a novel cDNA from HL-60 cells treated with 1,25-dihydroxyvitamin D-3. Biochim Biophys Acta 1994;1219:26–32.
[10] Junn E, Han SH, Im JY, et al. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol 2000;164:6287–95.
[11] Yamawaki H, Berk BC. Thioredoxin: a multifunctional antioxidant enzyme in kidney, heart and vessels. Curr Opin Nephrol Hypertens 2005;14:149–53.
[12] World CJ, Yamawaki H, Berk BC. Thioredoxin in the cardiovascular system. J Mol Med 2006;84:997–1003.
[13] Minn AH, Couto FM, Shalev A. Metabolism-independent sugar effects on gene transcription: the role of 3-O-methylglucose. Biochemistry 2006;45:11047–51.
[14] Minn AH, Pise-Masison CA, Radonovich M, et al. Gene expression profiling in INS-1 cells overexpressing thioredoxin-interacting protein. Biochem Biophys Res Commun 2005;336:770–8.
[15] Yamawaki H, Pan S, Lee RT, Berk BC. Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. J Clin Invest 2005;115:733–8.
[16] Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, et al. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. Circulation 1994;90:583–612.
[17] McKenzie KJ, Crowcroft NS. Race, ethnicity, culture, and science. BMJ 1994;309:286–7.
[18] Moser M. World Health Organization – International Society of Hypertension Guidelines for the Management of Hypertension—do these differ from the U.S. recommendations? Which guidelines should the practicing physician follow? J Clin Hypertens (Greenwich) 1999;1:48–54.
[19] Alvim RO, Freitas SR, Ferreira NE, et al. APOE polymorphism is associated with lipid profile, but not with arterial stiffness in the general population. Lipids Health Dis 2010;9:128.
[20] Campos LC, Miyakawa AA, Barauna VG, et al. Induction of CRP3/MLP expression during vein arterialization is dependent on stretch rather than shear stress. Cardiovasc Res 2009;83:140–7.
[21] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
[22] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[23] Coon H, Singh N, Dunn D, et al. TXNIP gene not associated with familial combined hyperlipidemia in the NHLBI Family Heart Study. Atherosclerosis 2004;174:357–62.
[24] van Greevenbroek MM, Vermeulen VM, Feskens EJ, et al. Genetic variation in thioredoxin interacting protein (TXNIP) is associated with hypertriglyceridaemia and blood pressure in diabetes mellitus. Diabet Med 2007;24:498–504.
[25] Parikh H, Carlsson E, Chutkow WA, et al. TXNIP regulates peripheral glucose metabolism in humans. PLoS Med 2007;4:e158.
[26] Shalev A, Pise-Masison CA, Radonovich M, et al. Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. Endocrinology 2002;143:3695–8.
[27] Mochizuki M, Kwon YW, Yodoi J, Masutani H. Thioredoxin regulates cell cycle via the ERK1/2-cyclin D1 pathway. Antioxid Redox Signal 2009.
[28] Schulze PC, Yoshioka J, Takahashi T, et al. Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein. J Biol Chem 2004;279:30369–74.
[29] Buckle A, Dunn L, Ng MKC. Hyperglycaemia inhibits thioredoxin-mediated angiogenesis: implications for impairment of neovascularization in diabetes mellitus. Heart Lung Circ 2007;16:S214–5.