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Mn-SOD 47 CC genotype in combination with high tea consumption may prevent complications in Tunisian type-2 diabetes

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ABSTRACT. Reactive oxygen species metabolizing enzymes may play an important role in the prevention of type-2 diabetes (T2D) complications. We analyzed the association between Cu/Zn-SOD +35 A/C, Mn-SOD T47C, and CAT -21 A/T gene polymorphisms and complications, in combination with tea consumption in Tunisian T2D. A sample of 366 T2D subjects was enrolled in this study. All participants were asked about tea consumption and frequency. Anthropometric, clinical, and routine biochemical characteristics were obtained from subjects' updated medical records. Malondialdehyde, as an early marker of lipid peroxidation, was measured in plasma samples. Urinary polyphenol derivatives (UPDs), as a marker of polyphenols intake, were assessed by the Folin-Ciocalteu assay. SODs and CAT

genotypes were determined by conventional restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) methods. From all subjects, the results showed that in high tea consumers (>3 cups/day), the frequency of the Mn-SOD 47 CC genotype was significantly higher in T2D without complications compared with T2D with complications (P = 0.03; OR = 0.284; 95%CI = 0.086-0.939). However, no significant associations were observed with Cu/Zn-SOD +35 A/C or CAT -21 A/T genes polymorphisms. Additionally, the evaluation of UPDs showed that individuals carrying the Mn-SOD 47 CC genotype and consuming more than three cups of tea per day present significantly higher UPDs (P = 0.038). In conclusion, the Mn-SOD 47 C variant in combination with high tea consumption may provide protection against complications in T2D.

Key words: SODs and CAT polymorphisms; Tea intake; Urinary polyphenol derivatives; Type-2 diabetes complications; T2D complications

INTRODUCTION

As with other multifactorial diseases, type 2 diabetes (T2D) is mostly the result of interaction between both genetic and clinical factors such as oxidative stress (Villeneuve and Natarajan, 2010). Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, including enzymatic and non-enzymatic systems (Poljsak et al., 2013). Implication risk of T2D is closely associated with oxidative stress, which is a link between disease and long-term diabetes complications (Aditi et al., 2013). There is evidence indicating that oxidative stress is associated with renal damage, cardiovascular morbidity, and mortality (Ascencio-Montiel et al., 2013). The antioxidant enzyme pathway is the primary defense against ROS-induced damage (Guérin et al., 2001). Genetic variations in the antioxidant genes coding for the superoxide dismutase (SOD), CAT, and GPX enzymes may impair the regulation of enzymatic activities and ROS elimination. Thus, genetic variations in antioxidant enzymes could modulate disease risk (Forsberg et al., 2001). The potential involvement of SOD and CAT polymorphisms in susceptibility risk of developing diabetes and its complications has been reported elsewhere, but not in Tunisian T2D (Dutkiewicz et al., 2010; Karič et al., 2012). A meta-analysis showed a significant association of the T47C C allele of Mn-SOD with reduced risk of diabetic microvascular complications including diabetic nephropathy, diabetic retinopathy, and diabetic polyneuropathy in dominant, recessive, and codominant models (Tian et al., 2011). The SODs are a class of enzymes involved in the detoxification of superoxide into hydrogen peroxide (Montano et al., 2012). Three major isoforms of SOD have been identified according to their tissue location and transition metals. Cu/Zn-SOD essentially resides in the cytoplasm, Mn-SOD in the mitochondria, which is the major site for the manufacture of ROS, and ecSOD is extracellular (Neves et al., 2012; Crawford et al., 2012). CAT regulates the metabolism of hydrogen peroxide by decomposing it into water and oxygen (Zhang et al., 2011).

Nowadays, more attention is focused on tea consumption because both green and black teas contain numerous ingredients, such as polyphenolic compounds, that enable better

control of the T2D pathway, especially insulin resistance (Bahadoran et al., 2013). In previous meta-analyses of the EPIC-InterAct case-cohort study conducted in 26 centers in eight European countries and consisting of a total of 16,835 T2D individuals, tea consumption was inversely associated with incidence of T2D. Indeed, a linear inverse association was observed between tea consumption and incidence of T2D. People who drink at least four cups of tea per day may have a 16% lower risk of developing T2D than non-tea drinkers (InterAct Consortium et al., 2012). Therefore, an investigation of the beneficial effects of the combination of polymorphism in antioxidant enzymes and tea consumption could have great practical importance in medical T2D care.

The purpose of the current study was to assess the association between Cu/Zn-SOD, Mn-SOD, and CAT gene polymorphisms [+35 A/C in the Cu/Zn-SOD gene (rs2234694), T47C in the Mn-SOD gene (rs4880), and -21 A/T in the CAT gene (rs7943316)] and T2D complications in moderate and high tea consumers. In addition, we investigated whether these allelic variants of the SOD and CAT genes were correlated with levels of urinary polyphenol derivatives (UPDs) (a marker of polyphenols intake) in moderate and high consumers of tea.

MATERIAL AND METHODS

Patients

This study enrolled a total of 366 adults with T2D. Patients were recruited randomly from different consulting services of the National Institute of Nutrition in Tunis. This research was approved by the local Ethics Committee and written consent was obtained from patients. Anthropometric, clinical, and biochemical characteristics, and types of complication or associated pathologies were obtained from the updated medical records of all subjects. For tea consumption, all participants were asked about the type of tea (green or black) and the number of cups consumed daily (mean cup serving, 35 mL). Green tea was majorly consumed (up to 80% of cases) in the form of a decoction + sugar or sweetener, which is a popular beverage in Tunisia and in other North African countries. It has been demonstrated that decoctions from green or black tea provide high polyphenols content (Dhaouadi et al., 2010). The green tea decoction was characterized by the brewing of the dried tea leaves in boiling water for a variable period of time, not exceeding 60 min overall. However, tea infusions consumed in most Western countries are prepared by adding a proportion of ~1 g leaf to 100 mL hot water and letting it brew for about 3 min. A subdivision of subjects was performed according to tea intake frequency: moderate tea consumers (<3 cups/day, N = 226) and high consumers (>3 cups/day, N = 140).

Biochemical analyses

Patients' routine biochemical parameters were identified from their updated medical records. Blood (3-5 mL) was collected in an ethylenediaminetetraacetic acid-containing tube. The plasma obtained was used for the determination of malondialdehyde assay (MDA), as an early marker of lipid peroxidation, according to the Ohkawa et al. (1979) method. Additionally, about 5 mL urine was collected to measure UPDs. The extraction of polyphenols from urine was performed according to the method used by Dhaouadi et al. (2010). UPD level was estimated spectroscopically by the Folin-Ciocalteu assay described by Singleton and Rossi (1965).

Genotyping

Genomic DNA was isolated from blood samples, taken in ethylenediaminetetraacetic acid, using an innuPREP Blood DNA Mini Kit (Analytik Jena AG, Thuringia, Germany). Determination of the SOD and CAT polymorphisms was achieved by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) analysis using the primers and the restriction enzymes shown in Table 1. PCR was carried out in a final volume of 25 µL, using 1 µL 100 ng genomic DNA, 0.2 U Taq DNA polymerase (Analytik Jena AG), 2.5 µL 10X buffer, 0.2 mM dNTP, 0.4 µM of each of the primers, and 1.5 mM $MgCl_2$. PCR cycles were carried out in a DNA Thermal Cycler (MultiGene, Labnet) according to the following program: an initial denaturation of DNA at 94°C for 5 min; 35 cycles of 1 min at 94°C; 30 s at 59°C, or 57°C, or 60°C to detect Cu/Zn-SOD +35 A/C, Mn-SOD T47C, or CAT -21 A/T polymorphisms, respectively; and 45 s at 72°C. A final extension was performed at 72°C for 7 min. The resulting PCR products were digested with the corresponding restriction endonucleases: 5 U *Hha*I (Thermo Scientific), 2.5 U *Bsa*wI (New England BioLabs), and 5 U *Hin*fI (Jena Bioscience) according to the manufacturer recommendations. Digestion products were analyzed electrophoretically on 3% agarose gel stained with ethidium bromide.

Table 1. Primers and restriction fragment length polymorphism-polymerase chain reaction conditions used for detection of the Cu/Zn SOD +35 A/C, Mn-SOD C47T, and CAT -21 A/T polymorphisms.

Polymorphism	Primers	Restriction enzymes	Annealing temperature	Restriction fragments
Cu/Zn SOD + 35 A/C	5'-CTATCCAGAAAACACGGTGGGCC-3' 5'-TCTATATTCAATCAAATGCTACAAAAC-3'	*Hha*I	59°C	C allele 71 and 207 bp A allele 278 bp
Mn-SOD C47T	5'-GCTGTGCTTTCTCGTCTTCAG-3' 5'-TGGTACTTCTCCTCGGTGACG-3'	*Bsa*wI	57°C	C allele 267 bp, T allele 183 and 84 bp
CAT -21 A/T	5'-AATCAGAAGGCAGTCCTCCC-3' 5'-TCGGGGAGCACAGAGTGTAC-3'	*Hin*fI	60°C	A allele 203 and 47 bp T allele 250 bp

Statistical data

Quantitative data are reported as mean ± standard deviation and were compared using the unpaired Student *t*-test analysis. Qualitative data are expressed in percentages and were compared using the χ^2 test. Genotype frequency differences in Cu/Zn-SOD +35 A/C [CC/C carriers *vs* AA (reference group)], Mn-SOD T47C [CC/C carriers *vs* TT (reference group)], and CAT -21 A/T [TT/T carriers *vs* AA (reference group)] between groups were examined by the Pearson χ^2 test. ORs with their corresponding 95%CI were calculated. Polyphenol excretion was compared in the different groups between genotypes of Cu/Zn-SOD +35 A/C [CC/C carriers *vs* AA (reference group)], Mn-SOD T47C [CC/C carriers *vs* TT (reference group)], and CAT -21 A/T [TT/T carriers *vs* AA (reference group)] using the unpaired Student *t*-test. A $P < 0.05$ was considered to be statistically significant. All statistical analyses were undertaken using the SPSS version 17 software.

RESULTS

Comparisons between green tea consumption groups in term of biological parameters are shown in Table 2. The biological parameters showed significant differences in the markers

of oxidative stress: uric acid (P = 0.04) and MDA (P < 0.01), which significantly decreased among the high consumers group (N cups > 3). UPD was significantly higher among high consumers (P = 0.03).

The genotype distribution of Cu/Zn-SOD +35 A/C, Mn-SOD T47C, and CAT -21 A/T according to diabetes complications and tea consumption are presented in Table 3. Among the high tea consumers (N cups >3) subgroup, the Mn-SOD T47C CC genotype frequency was higher in non-complicated diabetics (0.13) compared with complicated diabetics (0.32, P = 0.03; OR = 0.284; 95%CI = 0.086-0.939). However, no differences were observed in the genotypic distribution of Cu/Zn-SOD +35 A/C [CC *vs* AA and (CC+CA) *vs* AA] and CAT -21 A/T [TT *vs* AA and (TT+TA) *vs* AA] between complicated and non-complicated T2D.

The UPD comparison between Cu/Zn-SOD +35 A/C, Mn-SOD T47C, and CAT -21 A/T genotypes is shown in Table 4. No statistical differences were revealed in the distribution of UPD levels between genotypes tested, excepting in the high tea consumers subgroup. Among the latter, the Mn-SOD T47C CC genotype-carrying individuals showed a significantly higher UPD level (4.77 ± 3.42 mg/100 mL) compared with TT genotype-carrying subjects (3.79 ± 3.09 mg/100 mL, P = 0.038).

Table 2. Anthropometric, clinical, and biochemical characteristics of subjects based on tea intake.

	Patients (N = 366)	Tea consumption		P
		N cups < 3 (N = 226)	N cups > 3 (N = 140)	
Age (years)	54.33 ± 9.38	54.05 ± 9.42	54.89 ± 9.32	-
Gender (M/F)	153/213	86/140	67/73	-
BMI (kg/m^2)	30.41 ± 5.39	30.41 ± 5.44	30.41 ± 5.30	-
Current smoker				-
Yes (%)	17.5	16.7	18.9	
No (%)	82.5	83.3	81.2	
Duration of diabetes (years)	10.18 ± 6.62	11.05 ± 6.34	10.45 ± 7.21	-
Physical activity				-
Yes (%)	31.7	33.1	28.9	
No (%)	68.3	66.9	71.1	
Treatment				-
Insulin (%)	78.2	79.9	74.3	
Oral hypoglycemic drugs (%)	11	8.7	15	
Combination of both (%)	10.8	11.4	10.7	
Complication				
Yes [N (%)]	101 (27.6)	61 (27)	40 (28.5)	
No [N (%)]	265 (72.4)	165 (73)	100 (71.5)	0.72
Complication type				
Retinopathy [N (%)]	80 (79.2)	46 (75.4)	34 (85)	0.25
Nephropathy [N (%)]	23 (22.7)	15 (24.6)	8 (20)	0.72
Neuropathy [N (%)]	9 (8.9)	4 (6.5)	5 (12.5)	0.3
Systolic blood pressure (mmHg)	13.49 ± 2.13	13.5 ± 2.08	13.47 ± 2.24	0.91
Diastolic blood pressure (mmHg)	7.99 ± 1.19	8.04 ± 1.12	7.9 ± 1.32	0.27
Protein (g/L)	69.95 ± 9.16	70.43 ± 9.18	68.97 ± 9.09	0.15
HbA_{1c} (%)	9.14 ± 1.81	9.09 ± 1.83	9.26 ± 1.77	0.4
Glucose (mM)	11.36 ± 3.1	11.28 ± 3.12	11.52 ± 3.08	0.49
Total cholesterol (mM)	4.89 ± 0.92	4.9 ± 0.85	4.87 ± 1.05	0.79
LDL cholesterol (mM)	2.98 ± 0.77	2.98 ± 0.71	2.99 ± 0.87	0.89
HDL cholesterol (mM)	1.22 ± 0.43	1.23 ± 0.44	1.22 ± 0.41	0.84
Triglycerides (mM)	1.5 ± 0.78	1.51 ± 0.78	1.49 ± 0.78	0.76
Creatinine (µM)	75.37 ± 19.37	74.97 ± 19.83	76.2 ± 18.44	0.56
Uric acid (µM)	250.77 ± 73.37	256.04 ± 75.45	240.13 ± 68.05	0.04*
MDA (mM)	4.2 ± 1.97	5.31 ± 2.13	3.03 ± 1.7	<0.01**
UPD (mg/100 mL)	3.49 ± 2.56	3.16 ± 2.29	3.8 ± 2.83	0.03*

*(P < 0.05); **(P < 0.01).

Table 3. Cu/Zn SOD, Mn-SOD, and CAT genotypes according to diabetes complication and tea consumption.

	All (N = 366)			Tea cups < 3 (N = 226)			Tea cups > 3 (N = 140)		
Cu/Zn SOD genotypes	AA	CA	CC	AA	CA	CC	AA	CA	CC
Without complication (N = 265) (%)	181 (0.68)	59 (0.22)	25 (0.09)	113 (0.68)	34 (0.22)	18 (0.1)	68 (0.68)	25 (0.25)	7 (0.07)
With complication (N = 101) (%)	72 (0.71)	21 (0.20)	8 (0.08)	43 (0.7)	12 (0.19)	6 (0.1)	29 (0.72)	9 (0.22)	2 (0.05)
P		0.58[b]	0.61[a]		0.77[b]	0.79[a]		0.6[b]	0.62[a]
Mn-SOD genotypes	CC	CT	TT	CC	CT	TT	CC	CT	TT
Without complication (N = 265) (%)	79 (0.29)	139 (0.54)	47 (0.17)	47 (0.28)	91 (0.55)	27 (0.17)	32 (0.32)	48 (0.48)	20 (0.20)
With complication (N = 101) (%)	25 (0.25)	52 (0.51)	24 (0.24)	20 (0.33)	28 (0.46)	13 (0.21)	5 (0.13)	24 (0.6)	11 (0.27)
P	0.15[c]	0.19[d]		0.77[c]	0.38[d]		0.03[c]*	0.33[d]	
CAT genotypes	AA	AT	TT	AA	AT	TT	AA	AT	TT
Without complication (N = 265) (%)	64 (0.24)	143 (0.54)	58 (0.21)	43 (0.26)	83 (0.50)	39 (0.23)	21 (0.21)	60 (0.60)	19 (0.19)
With complication (N = 101) (%)	24 (0.23)	51 (0.50)	26 (0.26)	18 (0.29)	27 (0.44)	16 (0.26)	6 (0.15)	24 (0.60)	10 (0.25)
P		0.93[f]	0.59[e]		0.6[f]	0.96[e]		0.41[f]	0.31[e]

[a]CC *vs* AA; [b](CC+CA) *vs* AA; [c]CC *vs* TT; [d](CC+CT) *vs* TT; [e]TT *vs* AA; [f](TT+AT) *vs* AA; *P < 0.05; odds ratio (OR) = 0.284; 95% confidence interval (95%CI) = 0.086-0.939.

Table 4. Comparison of urinary polyphenol derivative levels (mg/100 mL) between Cu/Zn SOD, Mn-SOD, and CAT genotypes according to tea consumption.

Genotypes	Patients (N = 366)	P	Tea consumption			
			N cups < 3 (N = 226)	P	N cups > 3 (N = 140)	P
Cu/Zn SOD						
AA	3.19 ± 2.27		2.84 ± 2.03		3.56 ± 2.51	
CC	3.67 ± 2.41	0.342	3.31 ± 2.05	0.352	4.02 ± 2.79	0.307
CC+AC	3.12 ± 2.56	0.815	2.83 ± 2.3	0.914	3.44 ± 2.72	0.696
Mn-SOD						
TT	3.51 ± 2.76		3.2 ± 2.34		3.79 ± 3.09	
CC	4.13 ± 3.26	0.082	3.54 ± 2.7	0.406	4.77 ± 3.42	0.038*
CC+CT	3.81 ± 2.88	0.36	3.37 ± 2.38	0.567	4.24 ± 3.31	0.286
CAT						
AA	3.69 ± 2.57		3.45 ± 2.28		3.93 ± 2.86	
TT	3.14 ± 2.2	0.239	2.86 ± 2.14	0.113	3.46 ± 2.27	0.353
TT+TA	3.22 ± 2.42	0.307	3.03 ± 2.28	0.284	3.49 ± 2.62	0.374

Cu/Zn SOD AA, Mn-SOD TT, and CAT AA genotypes are taken as reference groups. *P < 0.05.

DISCUSSION

SOD and CAT constitute the initial antioxidant defense system that removes toxic free radicals and maintains the redox balance (Laddha et al., 2013). Allelic variation in Cu/Zn-SOD has been implicated in human disorders, particularly kidney disease, diabetes, and hypertension (Heink et al., 2013). The +35 A/C polymorphism in Cu/Zn-SOD is adjacent to the splicing point (exon3/intron3) and is related to Cu/Zn-SOD activity with AA genotype having the higher activity (Panduru et al., 2010). Our data showed no association between Cu/Zn-SOD +35 A/C polymorphism and T2D complications. In contrast to type 1 diabetes (T1D), the association of this variation with T2D has been poorly documented. Panduru et al. (2010) illustrate an association of the C-mutant allele in the Cu/Zn-SOD gene with advanced

stages of diabetic nephropathy in patients with T1D in Romania. Other studies have found multiple associations between Cu/Zn-SOD single nucleotide polymorphisms (SNPs) and the persistence of micro albuminuria, and the prevalence of diabetic nephropathy and retinopathy in T1D subjects (Al-Kateb et al., 2008; Mohammedi et al., 2011).

With regard to the CAT gene and T2D, one of the widely discussed catalase gene polymorphisms, CAT -21 A/T, which is located in the promoter region just proximal to the transcription start site, was investigated in our population. Our results did not show any relationship between CAT -21 A/T allelic variants and diabetes complication. In contrast, it has been reported that the mutant TT genotype is associated with hyperglycemia, hypercholesterolemia, and reduced catalase concentration, compared to the CC genotype in Hungarian T2D (Góth and Nagy, 2013).

In the Mn-SOD encoding gene, the T47C SNP, inducing the substitution from valine to alanine (V16A) at the 16th amino acid position in the second exon [also known as (V-9A) at position 9 in the signal peptide of human Mn-SOD], changes the structural conformation of the mitochondrial targeting sequence of the enzyme from an α-helix (A variant) to a β-sheet (V variant). This substitution may lead to misdirected intracellular trafficking inducing a 30-40% decrease in mitochondrial Mn-SOD activity and contributing to the accumulation of ROS (Chistyakov et al., 2001). It has been reported that the altered Mn-SOD variant increases the risk of oxidative stress associated with various pathologies, including diabetic microvascular disease (Chistyakov et al., 2001; Lee and Choi, 2006; Eras-Erdogan et al., 2009; Chen et al., 2012). Mn-SOD may be the most important member of the SOD family; therefore, people presenting Mn-SOD gene mutations are subjected to lower Mn-SOD efficiency and might be more prone to complications (Karič et al., 2012; Montano et al., 2012). In our study, we found that the Mn-SOD CC genotype may protect against the development of complications in Tunisian T2D. This result is consistent with those of Chistyakov et al. (2001) who found that V Mn-SOD was associated with diabetic neuropathy in a Russian population. It was observed that the TT genotype of this variant is associated with increased coronary heart disease risk in Caucasian females but not males with diabetes (Jones et al., 2010). Moreover, another study demonstrated that V Mn-SOD contributes to the development of diabetic retinopathy in Chinese T2D patients (Ye et al., 2008). However, these findings were not supported by Dutkiewicz et al. (2010) who did not observe an association of the V Mn-SOD gene with renal complications in diabetes mellitus. Furthermore, Lee and Choi (2006) have demonstrated that the V16A polymorphism of the Mn-SOD gene is not related to the development of diabetes and the progression of diabetic retinopathy, but is associated with diabetic macular edema in Korean T2D patients. A recent meta-analysis including 17 articles revealed that the C allele of the T47C polymorphism in the Mn-SOD gene protects against diabetic nephropathy and retinopathy (Tian et al., 2011).

We reported an Mn-SOD CC genotype protective effect against T2D complications only among a high tea consumer (N cups > 3) subgroup ($P = 0.03$; $OR = 0.284$; 95%CI = 0.086-0.939), whereas the association between the Mn-SOD CC genotype and complication progression was not significant in the patients studied as a whole ($P = 0.15$). This might be attributed to a combined effect of gene polymorphisms and tea intake in complication prevention. Indeed, the health benefits of tea consumption are well known (Khan and Mukhtar, 2007; Anhê et al., 2013).

In the high tea consumer group, uric acid and MDA were significantly lower than in the moderate tea consumer group, suggesting that high tea consumption is beneficial in the reduction of oxidative stress in T2D. The polyphenolic fraction of green tea was proposed as a possible candidate for diabetes prevention. Here, it is important to remember that most

polyphenolic compounds are present in food in the form of esters, glycosides, or polymers. It has to be acknowledged that these compounds must be converted to derivative metabolites in the intestinal lumen before they can be absorbed. During the course of absorption, polyphenols are conjugated in the small intestine and the liver (Manach et al., 2004). This process mainly includes methylation, sulfation, and glucuronidation, which restricts the toxic effects of polyphenol derivatives and facilitates their biliary and urinary elimination. Enterohepatic recycling may lead to a longer retention of derivative metabolites within the body (Manach et al., 2004). Polyphenol derivatives are able to penetrate tissues to exert their beneficial effects (van Duynhoven et al., 2011). Furthermore, an interaction between antioxidant enzyme genetic variants and tea polyphenols might explain this protective effect. Indeed, there have been many studies investigating the interactions between tea polyphenols and metabolizing enzymes. An administration of green tea polyphenols to alloxan diabetic rats indicated decreased lipid peroxidation associated with increased SOD and GST levels, whereas catalase was unchanged (Sabu et al., 2002). It has also been reported that the incubation of cultured rat brain astrocytes with catechins increases SOD (Cu/Zn-SOD and Mn-SOD subtypes) expression and activity (Chan et al, 2002). Similarly, Mori and Hasegawa (2003) have demonstrated that an addition of green tea to adipocytes enhances SOD activity.

In our study, we also analyzed the association between the metabolizing enzyme gene polymorphisms Cu/Zn-SOD +35 A/C, Mn-SOD T47C, and CAT -21 A/T and UPD rates, as a marker of polyphenols intake. To our knowledge, this is the first study examining the association between UPD, SOD, and CAT polymorphisms in relation to T2D complications. No straight association between UPD and diabetic complications was established ($P > 0.05$) (data not shown). However, genotype analysis only showed significantly higher UPD levels in the Mn-SOD T47C CC genotype-carrying individuals in the high tea consumers group ($P = 0.038$), whereas significance was not reached in patients as a whole ($P = 0.082$). Such interactions between UPDs and metabolizing enzymes in relation to disease risk have been investigated previously. Luo et al. (2012) found that the inverse association between flavonols and epigallocatechin in urine and breast cancer risk only appeared among those null for glutathione S transferase (GST) GSTM1, particularly null for both GSTM1 and GSTT1. The authors explained that for those null for GSTM1 and GSTT1 genes, the epigallocatechin may alternatively activate other cancer inhibitory GST forms. Another explanation could be that polyphenols may be more slowly excreted in urine among those null for GSTM1 and GSTT1, leading to higher bioavailability levels of cancer inhibitory tea polyphenols. With regard to Mn-SOD, it was established that its expression in mitochondria is upregulated by several polyphenolic compounds. Priego et al. (2008) showed that trans-pterostilbene (trans-3,5-dimethoxy-4′-hydroxystilbene) and quercetin (3,3′,4′,5,6-pentahydroxyflavone) induced an increase in Mn-SOD gene expression in HT-29 tumor-growing cells via an SP1 transcription factor mechanism. Other data have shown that epicatechins from cocoa significantly reduce Mn-SOD acetylation and nitrotyrosilation, via interaction with SIRT3 protein, leading to enhanced enzymatic activity (Ramirez-Sanchez et al., 2013). The authors also reported that Mn-SOD gene expression is modulated by epicatechins through an SIRT1 nuclear factor mechanism. These interactions between tea polyphenols and the Mn-SOD enzyme may provide an explanation for our findings on enhanced polyphenol excretion among Mn-SOD 47 CC genotype-carrying individuals. A possible explanation is that highly activated Mn-SOD (due to the CC genotype), and the resulting reduced oxidative stress within mitochondria, induces rapid trafficking of polyphenols throughout the body, which may explain the higher polyphenol excretion.

Finally, our study showed that the Mn-SOD 47 CC genotype, in combination with the

beneficial effects of tea polyphenols, prevents the development of complications in T2D. In addition, we report higher urine polyphenols excretion in Mn-SOD 47 CC genotype-carrying individuals. According to our findings, UPD excretion is further enhanced by a specific genetic background, such as the presence of the Mn-SOD T47C variant described here.

Conflicts of interest

The authors declare no conflict of interest.

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