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An association study between catalase -262C > T gene polymorphism, sodium-lithium countertrasport activity, insulin resistance, blood lipid parameters and their response to atorvastatin, in Greek dyslipidaemic patients and normolipidaemic controls

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Abstract

This study attempted to examine the effect of a functional catalase gene polymorphism, *CAT-262C > T*, on sodium-lithium countertransport (Na-Li CT) activity, insulin resistance determined as the homeostasis model assessment index (HOMA-IR), blood lipid parameters (cholesterol, triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol, apolipoprotein B, apolipoprotein A-I) and their response to atorvastatin, in previously characterized Greek dyslipidaemic patients and normolipidaemic controls. Putative associations were examined by running univariate analyses with a general linear model, using age, sex, smoking and hypertension as covariates. While no statistically significant associations were detected between the *CAT-262C > T* polymorphism and either baseline values or their modulation by atorvastatin in the patient group, HOMA-IR values were significantly ($p = 0.028$) lower among *CAT-262CC* controls compared to their *T* allele carrier counterparts. A trend towards higher plasma triglyceride values among *CAT-262CC* genotypes was also detected, in both dyslipidaemic patients and normolipidaemic controls.

Keywords: *Sodium-lithium countertransport, insulin resistance, blood lipids, catalase gene polymorphism, atorvastatin.*

Introduction

Erythrocyte sodium-lithium countertransport activity (Na-Li CT), assayed *in vitro*, has been positively associated with essential hypertension, dyslipidaemia, insulin resistance and diabetic neuropathy [1]. The mechanism(s) underlying these associations remain largely unknown, as Na-Li CT has so far eluded structural and genetic characterization, despite intense efforts to the opposite [2]. By virtue of its location on the erythrocyte membrane, Na-Li CT is constantly exposed to an oxygen-rich environment and to the modifying interactions associated with it. Reports that Na-Li CT kinetics are affected by thiol alkylating agents [3], apolipoprotein E genotype [4] and, possibly, glutathione S transferase (GST) polymorphisms [5] are in line with a probable physiological role of oxidative stress in modulating Na-Li CT activity and could provide a molecular explanation, at least in part, as to the associationof the latter with the clinical conditions mentioned above. We have recently shown that treatment of dyslipidaemic patients with atorvastatin resulted in significant reduction of erythrocyte Na-Li CT activity [6]. This reduction was independent of atorvastatin's lipid lowering activity and was only correlated with a decrease in the patients' insulin resistance [6].

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Human catalase is a homotetramer, heme-containing enzyme which catalyses the breakdown of H_2O_2 to H_2O and O_2. Catalase is highly active in erythrocytes [7], attesting to its importance for the antioxidative defense of these cells. *CAT -262C > T* (rs1001179) is a common polymorphism located in the promoter region of the human catalase gene, affecting the binding of nuclear proteins as well as the basal gene expression of catalase in various cells in culture [8]. The *T* allele has been associated with higher levels of the enzyme in blood, but, curiously enough, with lower catalase activity in erythrocytes [9–12]. The same polymorphism has been associated with diabetic neuropathy in type 1 diabetic Russian patients [13] but not with susceptibility to type 1 diabetes [14]. On the other hand, blood catalase deficiency has been associated with diabetes in a number of studies [15,16] and so has Na-Li CT [17]. There are no reports that we are aware of on the association of *CAT -262C > T* polymorphism with either Na-Li CT or insulin resistance, although dos Santos et al. [18] examined recently the possibility of an association of the same polymorphism with diabetic complications in Brazilian patients of Caucasian origin with type 2 diabetes (T2D) and concluded that there is no effect. Data on the effect of *CAT -262C > T* on blood lipid parameters are similarly scarce; with the exception of a single—negative—report from Hungary involving, again, T2D patients [19], no other reference was found in the literature.

In this report we describe a retrospective study of the effect of the *CAT -262C > T* polymorphism on basal Na-Li CT activity, insulin resistance and blood lipid parameters, as well as on their change following a 12 week-long treatment with atorvastatin [6], in a small cohort of previously characterized Greek dyslipidaemic patients and normolipidaemic controls.

Materials and methods

A total of 54 Greek nationals of Greek descent, residing in northern Greece, were included in this study. All of them were examined in the outpatient clinic of the 1st Propedeutic Clinic of AHEPA University Hospital, Thessaloniki, from November 2004 to May 2006 and diagnosed with primary dyslipidaemia [plasma cholesterol (CHOL) > 6.22 mmol/l, low density lipoprotein cholesterol (LDL-C) > 4.14 mmol/l, triglycerides (TG) < 2.82 mmol/l]. Almost half of these patients also suffered from mild essential hypertension (140 mmHg ≤ S.B.P. < 160 mmHg, 90 mmHg ≤ D.B.P. < 100 mmHg). Further details regarding diagnosis, inclusion and exclusion criteria have been published elsewhere [6,20]. Treatment with atorvastatin consisted of 20 mg of the drug, p.o., once daily, for 12 weeks. A small group of 25 normolipidaemic, mainly spousal control individuals was also included in this study.

Table I. Demographic characteristics of the participants in this study.

	Dyslipidaemic patients	Normolipidaemic controls
Sample size, *n*	54	25
Age (years ± SD)	48.69 ± 1.91	45.56 ± 9.64
Sex	26 ♂, 28 ♀	14 ♂, 11 ♀
Smoking	44.4%	44.0%
Mild hypertension	53.7%	—
CAT -262C > T	*CC*: 38 (70.4%) *TC*: 15 (27.8%) *TT*: 1 (1.8%)	*CC*: 16 (64.0%) *TC*: 9 (36.0%) *TT*: — (0.0%)

Demographic and laboratory characteristics are presented in Table I. The principles of the Declaration of Helsinki were strictly adhered to in this study. Informed consent was obtained by all individuals included.

Na-Li CT activity was measured according to the original method developed by Canessa et al. [21], which determines Li^+ efflux from isolated erythrocytes in Na^+-free and Na^+-rich medium. Insulin resistance was determined by calculating the homeostasis model assessment index [HOMA-IR = fasting insulin (mU/l) × fasting glucose (mmol/l)/22.5]. Plasma CHOL, HDL-C and TG levels were determined by conventional enzymatic methods in a Hitachi 912 analyser. LDL-C was calculated according to the Friedewald et al. [22] equation. Apolipoprotein A-I (ApoA-I) and apolipoprotein B (apoB) were determined by immunoturbidometric methods (Turbiquant®, TurbiTime System, Dade Behring, Newark, DE).

Genomic DNA was isolated from peripheral blood leukocytes using a commercial DNA extraction kit (PureGene®, Gentra Systems, Plymouth, MN). *CAT -262C > T* genotyping was performed with a touched-down PCR method, followed by *Sma* I digestion of the amplified genomic DNA (Figure 1), as previously described [8].

Associations of *CAT -262C > T* with Na-Li CT activity, insulin resistance, blood lipid parameters and their modulation by atorvastatin were examined with

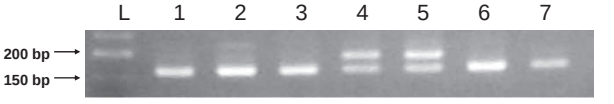

Figure 1. Agarose gel (2.5%) electrophoresis of *Sma*I digests following PCR amplification of the portion of the catalase gene harbouring the *CAT -262C > T* polymorphism. L: 100 bp ladder; 1,2,3,6,7: *CC*; 4,5: *CT*.

Table II. Distribution of sodium-lithium countertransport activity, insulin resistance, blood lipid parameters and their modulation by atorvastatin*, among dyslipidaemic patients following their stratification according to the *CAT-262C > T* genotype.

	All patients	*CAT-262CC*	*CAT-262TT, TC*	*p***
Na-Li CT (mmol $Li^+ \times l_{red\ blood\ cell}^{-1} \times h^{-1}$)	0.258 (0.236–0.286)	0.268 (0.239–0.296)	0.237 (0.194–0.280)	0.258
△Na-Li CT (mmol $Li^+ \times l_{red\ blood\ cell}^{-1} \times h^{-1}$)	−0.100 (−0.084–−0.117)	−0.098 (−0.077–−0.120)	−0.106 (−0.075–−0.137)	0.976
HOMA-IR (mU/l × mmol/l)	2.537 (2.107–2.966)	2.606 (2.014–3.197)	2.379 (1.782–2.975)	0.451
△HOMA-IR (mU/l × mmol/l)	−0.559 (−0.847–−0.271)	−0.518 (−0.904–−0.133)	−0.612 (−1.098–−0.135)	0.613
CHOL (mmol/l)	7.380 (7.155–7.555)	7.359 (7.130–7.589)	7.430 (7.078–7.780)	0.720
△CHOL (mmol/l)	−2.717 (−2.896–−2.525)	−2.695 (−2.932–−2.458)	−2.765 (−3.113–−2.436)	0.690
TG (mmol/l)	1.921 (1.743–2.071)	2.004 (1.806–2.203)	1.725 (1.391–2.059)	0.065
△TG (mmol/l)	−0.431 (−0.552–−0.313)	−0.466 (−0.614–−0.317)	−0.350 (−0.581–−0.119)	0.270
LDL-C (mmol/l)	5.168 (4.996–5.356)	5.141 (4.908–5.374)	5.232 (4.896–5.569)	0.473
△LDL-C (mmol/l)	−2.562 (−2.725–−2.396)	−2.533 (−2.758–−2.308)	−2.632 (−2.875–−2.388)	0.475
HDL-C (mmol/l)	1.320 (1.225–1.403)	1.285 (1.173–1.399)	1.403 (1.232–1.572)	0.315
△HDL-C (mmol/l)	0.019 (−0.027–0.075)	0.048 (−0.018–0.114)	−0.049 (−0.131–0.033)	0.088
apoB (mg/dl)	140.8 (134.6–146.8)	139.6 (131.6–147.6)	143.7 (132.4–154.9)	0.449
△apoB (mg/dl)	−46.5 (−52.6–−40.09)	−44.2 (−53.0–−35.4)	−52.0 (−59.7–−44.3)	0.130
apoA-I (mg/dl)	133.1 (126.2–139.0)	131.0 (123.3–138.8)	137.9 (124.1–151.7)	0.590
△apoA-I (mg/dl)	1.628 (−5.113–7.998)	2.463 (−6.512–11.448)	−0.356 (−10.264–9.552)	0.770

* As mean values (95% CI); ** between genotypes; Na-Li CT: Sodium-lithium countertransport activity; HOMA-IR: Homeostasis model assessment index; CHOL: total plasma cholesterol; TG: triglycerides; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; apoB: apolipoprotein B; apoA-I: apolipoprotein A-I; △ indicates change from baseline values following treatment of patients with atorvastatin.

an analysis of covariance programme with type III sums of square statistics, using age, sex, smoking and hypertension as covariates. All analyses were performed using the SPSS version 14.0 statistical package.

Results and discussion

The distribution of genotypes with respect to the *CAT -262C > T* polymorphism is shown in Table I. There was no statistically significant deviation from the Hardy-Weinberg equilibrium, with respect to either the dyslipidaemic patients ($p = 0.875$) or the controls ($p = 0.553$).

The distributions of our patients' Na-Li CT, HOMA-IR, blood lipid parameters as well as their change, in absolute values, following atorvastatin treatment, before and after stratification according to *CAT-262* genotype, is shown in Table II. The *CAT -262C > T* polymorphism appears to exert no strong effect, as there were no statistically significant differences between *CAT-262CC* genotypes and carriers of the *T* allele with respect to any of the parameters examined, with the possible exception of a trend ($p = 0.065$, Figure 2) towards higher baseline TG values among *CC* genotypes compared to *T* carriers. A similar trend was also observed among normolipidaemic controls ($p = 0.100$, Table III, Figure 2). This association approached statistical significance when the two groups were combined ($p = 0.053$), indicating that this may not be a chance finding and could reflect better protection from oxidative modification and an increased TG peripheral flow in *CC* genotypes. Another observation of probable significance is the apparent decrease of HOMA-IR values among normolipidaemic *CAT -262CC* genotypes compared to *T* carriers of the same group ($p = 0.028$, Table III, Figure 2). As *CAT -262CC* genotypes have been consistently associated with higher catalase activity in the past [9–12], our finding is well in agreement with the results of a recent study in which catalase transgenes were able to significantly prevent the development of experimentally-induced insulin resistance in cultured adipocytes [23]. We can only speculate as to why a similar effect was not detected among the patient group, but an inadequacy of the polymorphism-associated increase in catalase activity to cover the increased antioxidative demands of dyslipidaemic patients [24] may offer a conceivable explanation. Non-genetic parameters, including dietary habits, have been proposed to modify the effect of the *CAT -262C > T* polymorphism on erythrocyte catalase activity [10] and oxidative stress itself may modify these associations; while this manuscript was in preparation, D'souza et al. [25] reported that erythrocyte membrane-associated catalase is a major target of the lipid peroxidation product 4-hydroxylnonenal, an interaction which might conceivably offset gene polymorphism–activity associations, especially in dyslipidaemic patients. Indeed, while under normal conditions catalase functions as the predominant H_2O_2 removing enzyme [7], when its activity is impaired, other, no less efficient antioxidant systems appear to be taking over [26]. Similar considerations may apply to the lack of an observed effect with respect to Na-Li CT. On the other hand, a

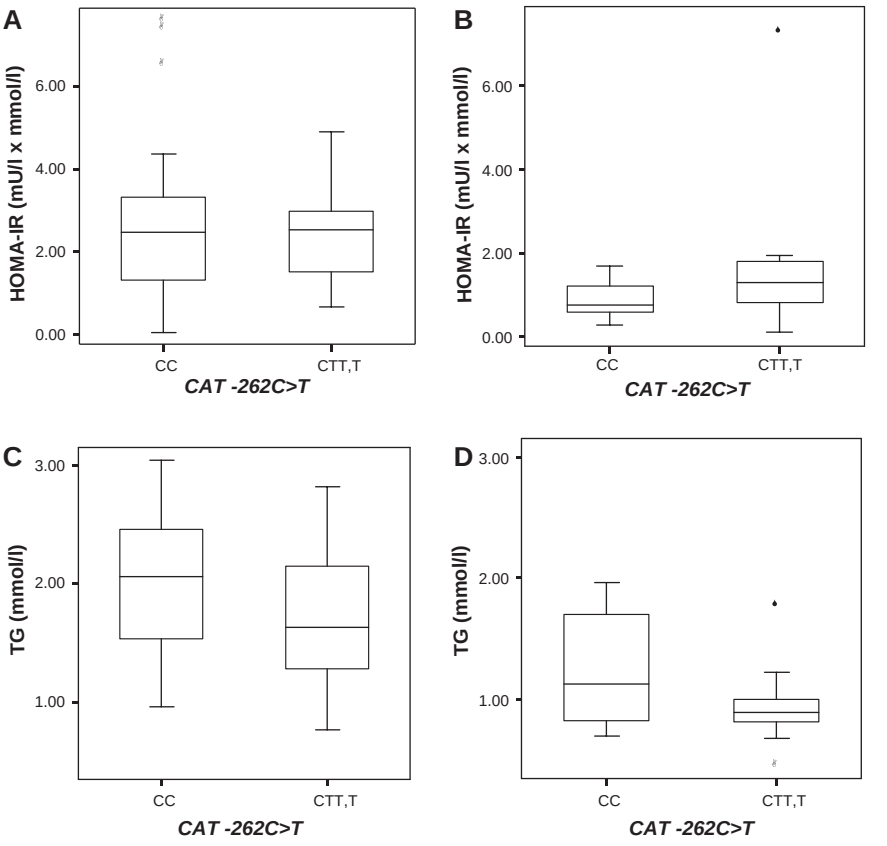

Figure 2. Distributions (box plots) of HOMA-IR and plasma TG values of dyslipidaemic patients (A, C) and normolipidaemic controls (B, D) following stratification according to *CAT-262C > T* genotype. Boxes: 25–75% interquartile range; horizontal bars: median values; whiskers: 95% confidence intervals; open circles: outliers; asterisks: extreme values.

direct effect of oxidative stress on Na-Li CT has not been documented in the past and—regrettably—no data on oxidative stress laboratory parameters were available in this retrospective study.

To the best of our knowledge, no study has attempted to examine a possible association between the *CAT-262C > T* gene polymorphism and either Na-Li CT or insulin resistance, in any group of patients, in the past. Similarly, very little, if anything, is known with respect to the effect of catalase or its gene polymorphisms on blood lipid parameters and their regulation by statins. While our results indicate that the *CAT-262C > T* gene polymorphism is unlikely to be a strong independent predictor of Na-Li CT, insulin resistance, blood lipids, or their response to atorvastatin, in dyslipidaemic patients, hints of an association worth pursuing in larger studies have emerged with respect to this polymorphism and HOMA-IR, but also TG. On the other hand, gene polymorphisms associated with the activity of other antioxidant systems could turn out to be more informative in that respect and should be examined in future studies.

Table III. Distribution of sodium-lithium countertransport activity, insulin resistance and blood lipid parameters*, among normolipidaemic controls, following stratification according to the *CAT-262C > T* genotype.

	All controls	*CAT-262CC*	*CAT-262TT, TC*	*p***
Na-Li CT (mmol $Li^+ \times l_{red\ blood\ cell}^{-1} \times h^{-1}$)	0.140 (0.118–0.161)	0.137 (0.101–0.173)	0.145 (0.122–0.168)	0.800
HOMA-IR (mU/l × mmol/l)	1.145 (0.657–1.636)	0.910 (0.687–1.132)	1.879 (0.264–3.494)	**0.028**
CHOL (mmol/l)	4.681 (4.515–4.846)	4.748 (4.567–4.928)	4.518 (4.033–5.003)	0.462
TG (mmol/l)	1.102 (0.944–1.259)	1.243 (1.014–1.473)	0.961 (0.673–1.248)	0.100
LDL-C (mmol/l)	2.605 (2.425–2.785)	2.629 (2.452–2.805)	2.418 (1.887–2.950)	0.468
HDL-C (mmol/l)	1.594 (1.447–1.742)	1.573 (1.391–1.756)	1.496 (1.222–1.771)	0.334
apoB (mg/dl)	87.3 (80.3–94.3)	91.6 (82.3–100.9)	85.4 (70.8–99.9)	0.610
apoA-I (mg/dl)	132.4 (121.1–143.7)	128.8 (114.1–143.4)	124.6 (104.1–145.0)	0.485

* As mean values (95% CI); ** between genotypes; Na-Li CT: Sodium-lithium countertransport activity; HOMA-IR: Homeostasis model assessment index; CHOL: total plasma cholesterol; TG: triglycerides; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; apoB: apolipoprotein B; apoA-I: apolipoprotein A-I; statistically significant differences in bold.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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