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Original article

Role of *CYP2E1* and *NQO1* polymorphisms in oxidative stress derived cancer in Thais with and without dyslipidemia

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Background: Hyperlipidemia can induce the endogenous production of reactive oxygen species (ROS), which may cause carcinogenesis. Cytochrome P450 (CYP)2E1 activity, induced by various factors including polyunsaturated fatty acids, effects the incidence of cancers, whereas NQO1, a flavoprotein, may protect against ROS.

Objectives: To investigate the effect of *CYP2E1* and *NQO1* polymorphism on oxidative stress status in Thais with and without dyslipidemia.

Methods: We included 1380 apparently healthy employees of the Electricity Generating Authority of Thailand in this study. We determined their *CYP2E1* and *NQO1* genotypes and related these to blood lipid profiles, and circulating levels of antioxidant enzymes, malondialdehyde (MDA), and reduced glutathione (GSH). Lifestyle-related factors were determined from questionnaires.

Results: All tested genotype frequencies were in Hardy–Weinberg equilibrium. The heterozygous and variant genotype distribution and allele frequency of *CYP2E1*5B* were less common than *CYP2E1*6*. Heterozygous *NQO1* was the most prevalent form. The frequency of the mutated allele *CYP2E1*5B* was 0.16, *CYP2E1*6* was 0.22, and *NQO1*2* was 0.43. Significant differences were observed for blood cholesterol, triglyceride, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol between normolipidemic participants, and those with hypercholesterolemia, hypertriglyceridemia, and combined hyperlipidemia. Participants in the hyperlipidemic subgroup who bore any variant alleles of genes had higher plasma MDA and GSH levels, and superoxide dismutase and glutathione peroxidase activity, but lower catalase activity when compared with normolipidemic participants bearing wild-type alleles.

Conclusions: Variations in genetic disposition and dyslipidemia can modify oxidative stress status. Relatively more free radicals may be generated in individuals in subgroups with hyperlipidemia bearing any variant alleles.

Keywords: *CYP2E1*, lipid profile, *NQO1*, oxidative stress, polymorphism

BMI = Body Mass Index, CAT = catalase, CYP = Cytochrome P450, GSH = reduced glutathione, GPx = glutathione peroxidase, Hb = hemoglobin, HDL-C = high-density lipoprotein-cholesterol, LDL-C = low density lipoprotein-cholesterol, MDA = malondialdehyde, NADPH = nicotinamide adenine dinucleotide phosphate, reduced form, NQO1 = NAD(P)H:quinone oxidoreductase 1, ROS = reactive oxygen species, SD = standard deviation, SE = standard error, SOD = superoxide dismutase.

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Oxidative stress can play a role in the pathogenesis of cancer [1]. Reactive species are generated endogenously, and from exogenous stimuli including xenobiotics. These reactive species are controlled by various cellular antioxidant systems [2, 3]. Hypercholesterolemia can induce the production of reactive oxygen species (ROS), such as superoxide anion, via enzymes including the oxidase for the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) and xanthine oxidase, and other sources of ROS from mitochondria [4]. Some investigators have reported an association between levels of plasma or serum lipids and lipoproteins, and various types of cancers [5-7]. Individual susceptibility to cancer can result from several host factors, especially differences in the activity of xenobiotic-metabolizing enzymes [8].

Cytochrome P450 (CYP)2E1-induced toxicity is apparently mediated by the activation of a state of oxidative stress by a wide variety of xenobiotics [9, 10]. The activity of CYP2E1 can be induced by ethanol, obesity, diabetes, and polyunsaturated fatty acids [10, 11]. At present, more than 14 *CYP2E1* genetic polymorphisms have been described [12], but only 3 point mutations have been extensively studied and shown to be linked to cancer risk [13]. Two point mutation polymorphisms of *CYP2E1* in 5′-flanking regions sensitive to the restriction enzymes *Pst*I (reference single-nucleotide polymorphism (SNP) rs3813867, G1293C) and *Rsa*I (rs2031920, C1053T), as revealed by restriction fragment length polymorphism (RFLP) analyses, are in complete linkage disequilibrium, and have been associated with high-transcription and increased enzyme activity [14]. Another polymorphism in intron 6, sensitive to *Dra*I, is T7632A (rs6413432) and is reported to enhance transcription of *CYP2E1* [15]. *CYP2E1* genetic polymorphisms effect the incidence of cancers [13].

Human NAD(P)H:quinone oxidoreductase 1 (NQO1) is a well-known phase II enzyme catalyzing diverse reactions that collectively result in broad protection against electrophiles, oxygen species, and superoxide anion radicals [16]. NQO1 also contributes to the maintenance of endogenous antioxidants [17]. The most widely studied *NQO1* SNP is a C609T transition in exon 6 of *NQO1* cDNA leading to 3 phenotypes: a wild-type phenotype with complete enzymatic activity, a heterozygous phenotype with around 3-fold decreased activity, and a homozygous mutant with only 2% to 4% full enzyme activity [18]. This SNP is rs1800566 in the National Center for Biotechnology Information database. To our knowledge, there are limited data on the association of hyperlipidemia and genetic polymorphisms of xenobiotic-metabolizing enzymes. Therefore, the present study sought to investigate the effect of genetic polymorphism of the 2 xenobiotic-metabolizing enzymes on the risk of cancer derived from oxidative stress in apparently healthy Thais with and without dyslipidemia, who have unintended daily exposure to various kinds of environmental chemicals.

Materials and methods

Study population

The study was approved by the Committee on Human Rights related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University (no. MURA2008/809/$S_{1\text{-}2}Aug_{10}$). We enrolled 1380 employees at the state enterprise Electricity Generating Authority of Thailand (EGAT) after written informed consent to participate in the study was obtained from each participant. Participants lived and worked in Bangkok, and had the possibility of unintended exposed to various kinds and doses of environmental chemicals. Participants completed a self-administered questionnaire, underwent a physical examination, and provided a fasting blood sample. We collected 20 mL of blood from each participant by venipuncture into ethylenediaminetetraacetic acid (EDTA)-containing and heparinized tubes that were immediately centrifuged at 2000*g*. Buffy coat, erythrocytes, and plasma were separated and stored at –20°C until genotyping and biochemical measurements were conducted.

Determination of reduced glutathione using Ellman's reagent

Whole blood (0.1 mL) was added to distilled water (1.9 mL) together with 3 mL of precipitating solution (100 mL containing 1.67 g glacial metaphosphoric acid, 0.2 g disodium EDTA, and 30 g sodium chloride). After standing for 5 min the mixture was filtered and filtrate (0.5 mL) was added to 0.3 M phosphate buffer, pH 6.4 (2 mL). Finally, we added 1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) in 1% sodium citrate (0.25 mL), mixed the solutions well, and within 4 min the absorbance of the mixture was read at 412 nm. We compared the absorbances with appropriate blanks without blood [19].

Determination of glutathione peroxidase

Whole blood (0.1 mL) was added to distilled water (1.9 mL) to make a 1:20 hemolysate. Then, prepared solutions of 1 M Tris-HCl, 5 mM EDTA, pH 8.0 (100 μL), 0.1 M GSH (20 μL), 10 U/mL glutathione reductase (100 μL), and 2 mM NADPH (100 μL)] were added to 10 μL of the hemolysate, together with 660 μL of distilled water and the mixture reaction preincubated at 37°C for 10 minutes. After the preincubation, the enzymatic reaction was initiated by addition of 10 L of freshly prepared 7 mM *t*-butyl hydroperoxide. The decrease in the optical density at 340 nm was measured against a blank for 1 min [20].

Determination of superoxide dismutase

Components of extraction solution (3.5 mL of cold distilled water, 1 mL of ethanol, 0.6 mL of chloroform) were added to 0.5 mL of hemolysate, then mixed for 1 min. After centrifugation at 3000 rpm for 10 minutes at 4°C, supernatant was collected to determine superoxide dismutase (SOD) activity. Seven serial dilutions were prepared for each sample. The final volume (3 mL) was composed of the following: 200 μL of 0.1 M EDTA containing 1.5 mg sodium cyanide, 100 μL of 1.5 mM nitroblue tetrazolium (NBT), 50 μL of 0.12 mM riboflavin, portion of sample (10, 20, 40, 60, 80, 200, or 500 μL) and 0.067 M potassium phosphate buffer, pH 7.8 to make a final volume. All tubes were illuminated in a light box for 12 min (18 W fluorescence) and the optical density of the mixtures was measured at 560 nm. The resulting inhibition of NBT reduction versus the amount of SOD extract was plotted on a linear scale. The amount of SOD that gave half of this maximum (1 unit) was determined from the plot [21].

Determination of catalase

Erythrocytes were diluted at the ratio of 1:500 in 50 mM phosphate buffer pH 7.0 (2.0 mL) to make a hemolysate. Then 30 mM H_2O_2 (1.0 mL) was added in a continuous stream from a pipette to promote mixing and to start the reaction. The decrease in the optical density of the mixtures was measured at 240 nm for 30 s following a previously described method [22].

Determination of malondialdehyde

Malondialdehyde (MDA) was determined using HPLC method with fluorescence detection [23]. The coefficient of variation was 4% within runs and 3% between days. The detection limit was 0.25 μmol/L and the method exhibited a linear response for MDA in range 1.5 to 15 μmol/L, the calibration curve presented a high correlation coefficient ($r^2 > 0.9$), $P = 0.001$; n = 10).

Determination of blood lipid profile

Serum cholesterol and triglyceride were analyzed using routine biochemical procedures at Ramathibodi Hospital. The classifications of lipid profiles were based on the criteria of the National Cholesterol Education Program [24]. Normolipidemia was defined as a triglyceride concentration <150 mg/dL and normal cholesterol concentration <200mg/dL. Dyslipidemias were defined as hypercholesterolemia (triglyceride <150 mg/dL, cholesterol >200 mg/dL), hypertriglyceridemia (triglyceride >150mg/dL, cholesterol <200mg/dL), and combined hyperlipidemia (triglyceride >150 mg/dL, cholesterol >200 mg/dL).

Genotype analysis

Genomic DNA was extracted from lymphocytes using a modified salting-out procedure [25] and frozen at –20°C until analysis. We conducted a TaqMan assay including a forward target-specific polymerase chain reaction (PCR) primer, a reverse primer, and TaqMan MGB probes labeled with a special dyes: FAM and VIC (Applied Biosystems, Waltham, MA, USA). The reaction mixture consisted of TaqMan Universal Master Mix (1×) and TaqMan-MGB probes for *CYP2E1*5B* (rs3813867, rs2031920), *CYP2E1*6* (rs6413432), and *NQO1* (rs1800566) in a total volume of 10 μL. The real-time PCR reaction protocol was 10 min at 95°C, 40 cycles of 15 s at 92°C, and 1 min at 60°C, using a 7500 Real-Time PCR System (Applied Biosystems). Data for specific probes and primers are available in the SNP500 database of the U.S. National Cancer Institute at *http://snp 500cancer.nci.nih.gov/* [26].

Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 19.0 (Armonk, NY, USA). All biochemical parameters (glutathione peroxidase (GPx), SOD, catalase (CAT), MDA, and reduced glutathione (GSH)) are expressed as geometric means with 95% confidence intervals (95% CIs) and means with standard deviations (SD). Goodness of fit to normal distribution was determined using a Kolmogorov–Smirnov test. Non-normally

distributed data was transformed into a log scale and retested for normal distribution before testing at the next step. A one-way analysis of variance and Mann–Whitney *U* test were conducted to compare difference in the means between 2 groups for normal and non-normally distributed data, respectively. A general linear model was applied to evaluate the significance of differences in the mean of parameters between more than 2 groups. Genotype frequencies were compared and tested for Hardy–Weinberg equilibrium using a Pearson χ^2 test. Logistic regression analyses were used to determine the association of oxidative stress markers with genes of interest and lipid profiles in the model adjusted for covariates (sex, smoking, and alcohol consumption). In this case, data were presented as an odds ratios (ORs) with 95% CIs. $P < 0.05$ was considered significant.

Results

Demographic characteristics of the study population

The demographic characteristics of 1380 EGAT employees (983 men and 397 women) were subdivided into 4 groups based on lipid profiles as summarized in **Table 1**.

Table 1. Demographic characteristics and distribution of the metabolic enzyme gene polymorphisms of the study population

Characteristics	Lipid profile				*P*[b]
	Normo-lipidemia (n = 236)	Hyper-cholesterolemia (n = 638)	Hyper-triglyceridemia (n = 84)	Combined hyperlipidemia (n = 422)	
Age (years)[a]	51.6 ± 4.7	51.0 ± 4.4	51.9 ± 4.8	51.9 ± 4.3	0.08
Weight (kg)[a]	67.1 ± 11.5	64.5 ± 10.8	69.3 ± 10.9	69.6 ± 10.6	0.46
Height (cm)[a]	164.4 ± 8.2	163.3 ± 7.6	164.3 ± 7.7	164.8 ± 7.5	0.16
BMI (kg/m^2)[a]	24.8 ± 3.48	24.1 ± 3.23	25.6 ± 3.38[c]	25.6 ± 3.41	<0.001[c]
Cholesterol (mg/dL)[a]	179.0 ± 19.12	242.6 ± 28.93[c]	180.1 ± 18.29	253.1 ± 35.80[d]	<0.001[c], 0.02[d]
Triglyceride (mg/dL)[a]	93.3 ± 32.40	98.9 ± 30.08	224.5 ± 95.70[c]	233.7 ± 105.32[d]	<0.001[c,d]
LDL-C (mg/dL)[a]	113.9 ±15.47	164.6 ± 30.13[c]	95.8 ± 19.12	159.4 ± 36.42[d]	<0.001[c], 0.003[d]
HDL-C (mg/dL)[a]	50.9 ±10.31	56.6 ± 11.56[c]	43.2 ± 9.93	47.3 ± 9.71	<0.001[c]
*CYP2E1:CYP2E1*5B* (*Rsa*I/*Pst*I)					
No. of c1c1 (WT)	175/176 (≈ 74)	450/453 (≈ 71)	54/55 (≈ 65)	300/300 (71)	<0.001[*]
No. of c1c2 (Het)	50/50 (21)	167/165 (≈ 26)	26/26 (31)	110/110 (26)	0.20, 0.25
No. of c2c2 (Var)	11/10 (≈ 4)	21/20 (≈ 3)	4/3 (≈ 4)	12/12 (3)	0.39, 0.29
*CYP2E1*6* (*Dra*I)					
No. of DD (WT)	148 (63)	380 (60)	48 (57)	263 (62)	<0.001[*]
No. of DC (Het)	74 (31)	224 (35)	30(36)	136 (32)	0.07
No. of CC (Var)	14 (6)	34 (5)	6 (7)	23 (6)	0.50
*NQO1 NQO1*2*					
No. of CC (WT)	71 (30)	210 (33)	30 (36)	127 (30)	0.10
No of CT (Het)	116 (49)	318 (50)	41(48)	215 (51)	<0.001[*]
No. of TT (Var)	49 (21)	110 (17)	13 (16)	80 (19)	0.41
Sex					
Male	170 (72)	419 (66)	63 (75)	331 (78)	<0.001[*]
Female	66 (28)	219 (34)	21 (25)	91 (22)	0.21
Smoking	33 (14)	64 (10)	11 (13)	82 (19)	<0.001[*]
Nonsmoking	203 (86)	574 (90)	73 (87)	340 (81)	0.001
Alcohol drinking	115 (49)	304 (48)	43 (51)	248 (59)	<0.001[*]
Alcohol abstinence	121 (51)	334 (52)	41 (49)	174 (41)	0.02[*]

BMI = Body Mass Index, Het = heterozygous, LDL-C = low density lipoprotein- cholesterol, HDL-C = high-density lipoprotein-cholesterol, Var = variant, WT = wild type

[a]Data = mean ± SD or n (%), [b]*P* generated using a general-linear model: multivariate test, χ^2 test for genotype and lifestyle features, [c]hypercholesterolemia or hypertriglyceridemia compare with normolipidemia, [d]combined hyperlipidemia compared with normolipidemia, [*]significant difference between group of lipid profiles

By comparison with normolipidemia, participants with different types of dyslipidemia (hypertriglyceridemia, hypercholesterolemia, and combined hyperlipidemia) showed significantly higher BMI, triglyceride, HDL-C, and LDL-C levels (all $P \leq 0.024$). The frequency of *CYP2E1*5B* c1c1 was approximately 71%, c1c2 was approximately 26%, and c2c2 was approximately 3%. The distribution of *CYP2E1*6* DD was 60%, DC was 34%, and CC was 6%. The distribution of *NQO1*2* CC was 32%, CT was 50%, and TT was 18%. The allele frequencies of *CYP2E1*5B*, *CYP2E1*6*, and *NQO1*2* were c1 0.84, c2 0.16; D 0.78, C 0.22; C 0.57, and T 0.43, respectively. All allele frequencies were in Hardy–Weinberg equilibrium in all lipid profile groups (all $P > 0.47$). Different blood lipid levels were found in participants who carried the wild-type *CYP2E1* (c1/c1) and (DD) alleles with $P < 0.001$. For *NQO1*, significant difference in blood lipid levels was found in participants who carried the heterozygous genotype (CT) with $P < 0.001$. The association of genetic variation and lipid profile on oxidative stress markers is presented in **Table 2**.

Table 2. Odds ratios for oxidative stress markers according to *CYP2E1* and *NQO1* polymorphism and lipid profile

Number (%)	Oxidative stress marker	Odds ratio (95% CI)[a]	*P*
CYP2E1: *Pst*I	Reference	1.0	
Wild-type	GSH	0.996 (0.997–1.02)	0.66
n = 984 (71.3)	CAT	0.73 (0.28–1.91)	0.52
• Heterozygous	SOD	0.58 (0.33–1.03)	0.06
n = 351 (25.4)	GPx	**2.53 (1.29–4.96)**	**0.007**
	MDA	1.24 (0.79–1.97)	0.35
• Variant	GSH	1.00 (0.96–1.05)	0.94
n = 45 (3.3)	CAT	6.49 (0.86–48.8)	0.07
	SOD	0.38 (0.09–1.54)	0.17
	GPx	3.33(0.65–17.15)	0.15
	MDA	0.81 (0.27–2.46)	0.72
CYP2E1: *Rsa*I	Reference	1.0	
• Wild-type	GSH	0.997 (0.98–1.02)	0.73
n = 979 (70.9)	CAT	0.60 (0.23–1.56)	0.29
• Heterozygous	SOD	0.59 (0.33–1.05)	0.07
n = 353 (25.6)	GPx	**2.58 (1.31–5.05)**	**0.006**
	MDA	1.27 (0.80–2.01)	0.31
• Variant	GSH	1.01 (0.97–1.05)	0.61
n = 48 (3.5)	CAT	**7.22 (1.05–48.8)**	**0.004**
	SOD	0.19 (0.05–0.77)	0.20
	GPx	2.34 (0.48–11.4)	0.24
	MDA	1.21 (0.43–3.52)	0.71
CYP2E1: *Dra*I	Reference	1.0	
• Wild-type	GSH	0.99 (0.97–1.01)	0.26
n = 839 (60.8)	CAT	0.68(0.28–1.65)	0.40
• Heterozygous	SOD	0.64 (0.38–1.09)	0.10
n = 464 (33.6)	GPx	1.79 (0.96–3.33)	0.07
	MDA	0.99 (0.79–1.97)	0.97
• Variant	GSH	0.99 (0.95–1.03)	0.58
n = 77 (5.6)	CAT	2.32 (0.42–13.0)	0.34
	SOD	0.78 (0.26–2.28)	0.64
	GPx	1.92 (0.53–6.90)	0.32
	MDA	1.08 (0.45–2.59)	0.86

Table 2. (Continuous) Odds ratios for oxidative stress markers according to *CYP2E1* and *NQO1* polymorphism and lipid profile

Number (%)	Oxidative stress marker	Odds ratio (95% CI)[a]	*P*
*NQO1*2*	Reference	1.0	
• Wild-type	GSH	1.02 (0.998–1.04)	0.08
n = 438 (31.7)	CAT	1.45 (0.57–3.66)	0.44
• Heterozygous	SOD	1.31 (0.75–2.28)	0.35
n = 690 (50.0)	GPx	0.90 (0.47–1.72)	0.74
	MDA	0.89 (0.56–1.40)	0.61
• Variant	GSH	1.02 (0.99–1.04)	0.19
n = 252 (18.3)	CAT	0.75 (0.22–2.53)	0.65
	SOD	1.09 (0.53–2.25)	0.81
	GPx	1.98 (0.84–4.64)	0.12
	MDA	1.15 (0.64–2.05)	0.65
Cholesterol <200 mg/dL	Reference	1.0	
n = 320 (23.2)			
Cholesterol ≥200 mg/dL	GSH	1.01 (0.99–1.03)	0.42
n = 1060 (76.8)	CAT	0.68 (0.26–1.79)	0.43
	SOD	0.85 (0.48–1.50)	0.57
	GPx	0.59 (0.30–1.17)	0.13
	MDA	**1.85 (1.14–2.98)**	**0.01**
Triglyceride <150 mg/dL	Reference	1.0	
n = 874 (63.3)			
Triglyceride	GSH	0.99 (0.98–1.01)	0.56
≥200 mg/dL	CAT	0.42 (0.18–1.01)	0.05
n = 506 (36.7)	SOD	1.13 (0.68–1.89)	0.64
	GPx	1.05 (0.57–1.93)	0.87
	MDA	**2.54 (1.66–3.89)**	**<0.01**

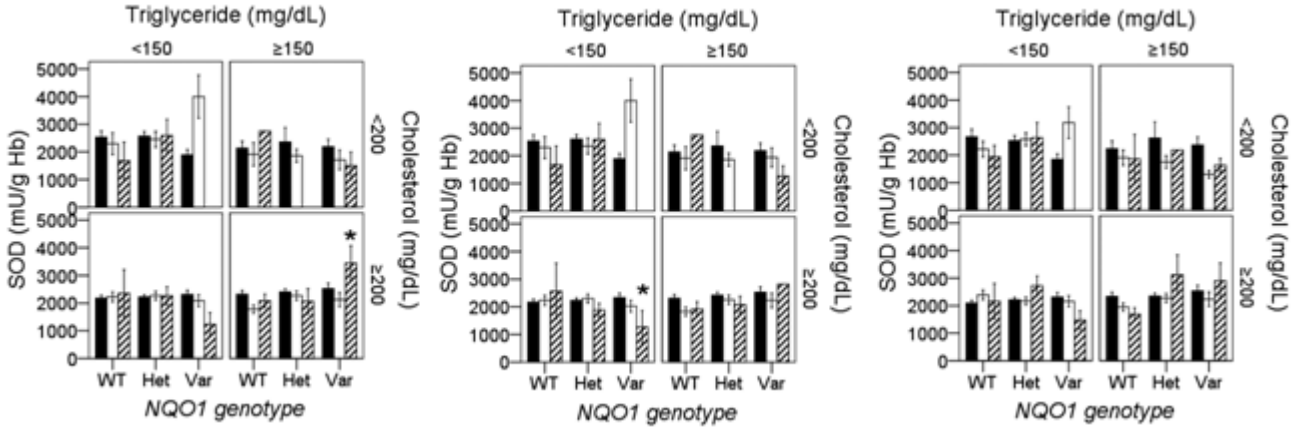
[a]Sex, smoking, and alcohol consumption were adjusted for odds ratio, GSH = reduced glutathione, GPx = glutathione peroxidase, SOD, superoxide dismutase, CAT = catalase, and MDA = malondialdehyde.

By comparison with the wild type, significant associations were found in heterozygous *CYP2E1 Pst*I with an OR 2.53 ($P = 0.007$)*/Rsa*I with an OR 2.56 ($P = 0.006$) for GPx and in variant *Rsa*I with an OR 7.22 ($P = 0.004$) for CAT. Significant associations between hyperlipidemia with MDA were observed (both $P \leq 0.01$). The combined effect of genetic variation and lipid profile on oxidative stress status was analyzed.

No significant differences in GSH level, CAT activity, or GPx activity were found between subgroups. However, the level of GSH (25.66 ± 8.66 mg/dL to 35.42 ± 1.76 mg/dL) tended to be higher in participants in the hyperlipidemia subgroup and participants bearing any variant alleles, than in participants bearing a wild-type allele with normolipidemia (28.00 mg/dL ± 6.52 to 32.66 ± 6.32 mg/dL).

Participants bearing any variant allele of either gene of interest tended to have a lower mean CAT activity (from 19.50 ± 9.95 kU/g hemoglobin (Hb) [95% CI] [6.5–39.06] to 37.17 ± 4.75 kU/g Hb [27.84–46.51]) than participants bearing the wild-type alleles of either gene of interest with normolipidemia (from 26.27 ± 1.68 kU/g Hb [22.97–29.57] to 37.06 ± 2.89 kU/g Hb [31.38–42.74]). Significant differences in SOD activity were found between the subgroups (**Figure 1A–C**) in combined hyperlipidemia subgroups and participants bearing any *NQO1* alleles with *Pst*I or *Rsa*I variants. Participants with homozygous *Pst*I or *Rsa*I variants had the highest mean SOD activity (1.69 ± 0.51 U/g Hb [0.70–2.69] to 3.45 ± 0.93 U/g Hb [1.62–5.27]) ($P = 0.01$ and $P = 0.03$).

Participants in the hyperlipidemia subgroup and participants bearing any variant allele of either gene of interest tended to have a higher mean GPx activity (31.61 ± 11.56 U/g Hb [95% CI] [8.9–54.33] to 52.29 ± 15.98 U/g Hb [19.91–82.66]) than participants bearing the wild-type alleles of both genes of interest with normolipidemia (25.24 ± 6.67 U/g Hb [12.13–38.35] to 42.40 ± 3.86 U/g Hb [34.82–49.98]) although the differences were not significant.



1A. pairing with *CYP2E1*5B* (*Pst*I) **1B. pairing with *CYP2E1*5B* (*Rsa*I)** **1C. pairing with *CYP2E1*5B* (*Dra*I)**

Figure 1A–C. Superoxide dismutase (SOD) activity (mU/g hemoglobin (Hb)) in participants with various *CYP2E1* genotypes (**A**) *Pst*I, (**B**) *Rsa*I, and (**C**) *Dra*I (*solid bars* = wild type, *open bars* = heterozygous, *hatched bars* = variant) paired with *NQO1* genotypes (WT = wild type, Het = heterozygous, and Var = variant), and various blood lipid profiles. Design for each panel: upper left quadrant is normolipidemia, upper right quadrant is hypertriglyceridemia, lower left quadrant is hypercholesterolemia, and lower right quadrant is combined hyperlipidemia. *Dra*I denotes the allele with a polymorphic site recognized by this restriction enzyme localized in intron 6 at position T7632A (rs6413432). *Pst*I indicates the allele with a point mutation recognized by *Pst*I in the 5′-flanking region at G1293C (rs3813867) and *Rsa*I indicates the allele with a point mutation recognized by *Rsa*I in the 5′-flanking region at C1053T (rs2031920). Error bars ± SE. **P* < 0.05.

In the subgroup with combined hyperlipidemia, participants bearing any *NQO1* allele with a *Rsa*I variant showed significantly higher levels of MDA than participants bearing any *NQO1* with a wild-type *Rsa*I allele with $P = 0.021$. Participants bearing a heterozygous *NQO1* allele with a *Dra*I variant had a significantly higher level of MDA than participants bearing any *NQO1* with a wild-type *Dra*I allele ($P = 0.031$). Comparison of the subgroups of hyperlipidemia showed participants bearing any variant alleles had a higher mean level of MDA (6.04 ± 2.74 (SE) μmol/L [range] [0.65–11.40] to 16.22 ± 5.68 μmol/L [5.03–27.41]) than participants bearing a wild-type allele with normolipidemia (4.00 ± 3.49 μmol/L [–2.80–10.8] to 11.97 ± 2.55 μmol/L [6.95–16.98]) **(Figure 2A–C)**.

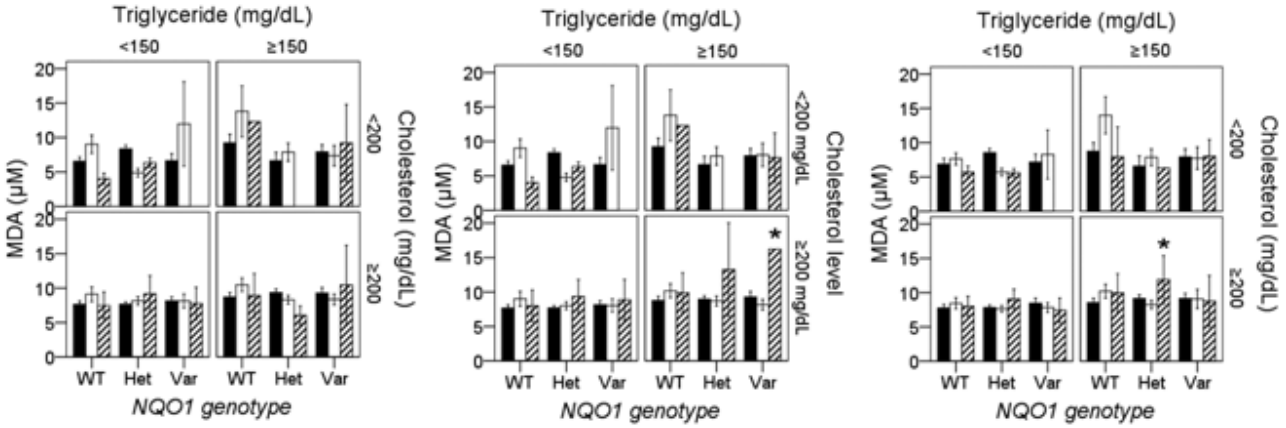
Discussion

The present study sought to investigate the impact of genetic polymorphism and dyslipidemia on oxidative stress, which is related to cancer risk. Oxidative stress status was assessed by measuring biomarkers of oxidative damage and indirectly assessing antioxidant defensive systems in blood samples.

Five biomarkers of oxidative stress status were determined in 1,380 healthy participants to assess associations of hyperlipidemia and genetic polymorphisms of drug or xenobiotic-metabolizing enzymes. An increase in free radical generation or a decrease in antioxidant levels in living organisms, or both, suggest that these factors play a critical role in the etiology of carcinogenesis [27]. Genetic variation was also considered a biomarker of susceptibility. Genetic variation is not only an indicator of susceptibility to chemical exposure, but also a modifier of several events in progression from exposure to disease [28]

In the study population, the heterozygous and variant genotype distribution and allele frequency of *CYP2E1*5B* was less common than *CYP2E1*6.* The heterozygous form of *NQO1* was the most prevalent found in this study. The frequency of the mutated allele *CYP2E1*5B* (c2) was 0.16 and *CYP2E1*6* (C) was 0.22, and these frequencies were close to frequencies reported in the Thai population [29]. The mutated allele of *NQO1*2* (T) was 0.43 and was close to that found in Asian populations [30].

In the present study, the participants in all hyperlipidemia subgroups and bearing any variant alleles of *CYP2E1*5B*, *CYP2E1*6*, or *NQO1*2* tended to have higher blood levels of MDA suggesting that more free radicals might be generated in



2A. pairing with *CYP2E1*5B* (*Pst*I) **2B. pairing with *CYP2E1*5B* (*Rsa*I)** **2C. pairing with *CYP2E1*5B* (*Dra*I)**

Figure 2A–C. Malondialdehyde (MDA) level (μM) in participants with various *CYP2E1* genotypes (**A**) *Pst*I, (**B**) *Rsa*I, and (**C**) *Dra*I (*solid bars* = wild type, *open bars* = heterozygous, *hatched bars* = variant) paired with *NQO1* genotypes (WT wild type, Het heterozygous, and Var variant), and various blood lipid profiles. Design for each panel: upper left quadrant is normolipidemia, upper right quadrant is hypertriglyceridemia, lower left quadrant is hypercholesterolemia, and lower right quadrant is combined hyperlipidemia. *Dra*I denotes the allele with a polymorphic site recognized by this restriction enzyme localized in intron 6 at position T7632A (rs6413432). *Pst*I indicates the allele with a point mutation recognized by *Pst*I in the 5′-flanking region at G1293C (rs3813867) and *Rsa*I indicates the allele with a point mutation recognized by *Rsa*I in the 5′-flanking region at C1053T (rs2031920). Error bars ± SE. **P* < 0.05.

individuals from any subgroup with hyperlipidemia bearing any variant alleles of the genes of interest. Hayashi et al. [31] reported a 10-fold higher transcription activity of the c2/c2 allele of *CYP2E1* compared with the c1/1c1 allele in a HepG2 cell line, suggesting that the transcription activity of the c2 allele must be more than that of c1. The c2 allele may mediate the susceptibility of an individual to oxidative stress.

Habitual smoking was a lifestyle feature of individuals with hypercholesterolemia and combined hyperlipidemia, as consistent with findings by Mari et al. [32]. Smoking with mild forms of hyperlipidemia was associated with an increase in some markers of oxidative stress. Interestingly, participants with hypercholesterolemia showed similar MDA levels irrespective of the genotype of the genes of interest and they also had a high level of HDL-C. These observations might be explained by the oxidative protection mechanism of HDL-C for LDL-C through HDL-associated enzymes, such as paraoxonase1, lecithin-cholesterol acyltransferase, or platelet-activating factor acetylhydrolase [33]. Alcohol consumption was presumably another cause of oxidative stress resulting in a tendency toward higher MDA levels in participants in any of the subgroups with hyperlipidemia bearing variant alleles of any gene of interest.

Alcohol consumption was also a lifestyle feature of participants with hypercholesterolemia or combined hyperlipidemia. CYP2E1 is endogenously induced by ethanol consumption and a variety of xenobiotics and ROS [9, 10]. Participants in all lipid profile subgroups carrying the wild-type *NQO1* allele with any allele of *CYP2E1* tended to have a low blood level of MDA, possibly because NQO1 is a radical scavenger [34].

Participants with hypercholesterolemia and combined hyperlipidemia had high levels of LDL-C. The polyunsaturated fatty acids in cholesterol esters, phospholipids, and triglycerides are subjected to free radical-initiated oxidation and can participate in chain reactions that may amplify the extent of the damage they cause. Aldehydes and ketones such as MDA are breakdown products polyunsaturated fatty acid oxidation [35]. Furthermore, all participants in this study were classified as being overweight (BMI >24 kg/m^2) [36, 37], which poses a risk of comorbidity and related diseases. Obesity may cause a chronic overproduction of ROS [38], which are metabolized by the network of enzymatic and nonenzymatic antioxidant systems [39].

Our present study assessed 4 different biomarkers of antioxidant status, including the activity of SOD, CAT, GPx, and the level of GSH in circulating blood. Inconsistent findings for the level of GSH and the activity of these antioxidant enzymes were observed in all lipid profile groups. In summary, participants in the hyperlipidemia subgroup bearing any variant allele of either gene of interest tended to have a higher GSH level, and SOD and GPx activity, but lower CAT activity when compared with participants bearing a wild-type allele with normolipidemia. The high level of GSH in the participants in the hyperlipidemia subgroup bearing any variant allele is probably because of upregulated GSH synthesis as a result of free radical exposure [40]. CYP2E1 may be induced by alcohol consumption and smoking by individuals in the subgroup with hyperlipidemia, which suggests that upregulation of GSH synthesis might be an adaptive response to attenuate CYP2E1-dependent oxidative stress [41]. The high activity of SOD and GPx might be explained in a similar manner. Gpx is an essential enzyme for all cell types under normal or low levels of oxidative stress. CAT therefore plays a more important role in protecting cells against severe oxidant stress when compared with GPx [42].

Conclusion

Our data suggest that the presence of any variant alleles of *CYP2E1* and *NQO1* associated with hyperlipidemia may attenuate antioxidant status and affect levels of oxidative stress markers. This finding provides data supporting the antioxidant status and oxidative stress marker involvement of endogenous (genetic variation and lipid profile) and exogenous factors (smoking and alcohol consumption) in interindividual variation. The variation in genetic background and dyslipidemia can modify oxidative stress, an issue of interest and motivation to extrapolate to the well-being practices of individuals in everyday life. From a toxicological point of view, the present study also provides information regarding gene–gene and gene–environment interaction.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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