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NADH/NADPH oxidase *p22 phox* C242T polymorphism and coronary artery disease in the Australian population

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Abstract

Background Oxidative stress induced by the superoxide anion ($\cdot O_2^-$) has been implicated in atherogenesis. The NADH/NADPH oxidase system is involved in $\cdot O_2^-$ production and *p22 phox* is an essential component of that system.

Material and methods We analysed the *p22 phox* C242T polymorphism in 689 consecutive Australian Caucasians aged ≤ 65 years with and without angiographically documented coronary artery disease (CAD)

Results We report the rare T allele frequency of 0·33, which is 3 fold higher than that reported in the Japanese population by Inoue *et al*. [7]. The genotype distributions were not different among patients with CAD (CC:0·422, CT:0·459 and TT: 0·119 in men; 0·447, 0·439 and 0·114 in women) and without CAD (0·479, 0·420 and 0·101%, $\chi^2 = 0{\cdot}794$, $P = 0{\cdot}672$ in men; 0·443, 0·471 and 0·86, $\chi^2 = 0{\cdot}442$, $P = 0{\cdot}802$ in women). The frequencies of the rare TT homozygotes or of the 'T' allele frequency were also not associated with the number of significantly stenosed vessels ($\chi^2 = 4{\cdot}466$, $P = 0{\cdot}614$ in men; $\chi^2 = 4{\cdot}736$, $P = 0{\cdot}578$ in women) or with a myocardial infarction (MI) history ($\chi^2 = 2{\cdot}310$, $P = 0{\cdot}315$ in men; $\chi^2 = 1{\cdot}178$, $P = 0{\cdot}555$ in women). However, when the analysis was conducted in young male patients aged ≤ 45 years ($n = 44$), TT + TC patients tended to have an increased risk for CAD (odds ratio: 5·71 95% CI: 1·22–26·75, $P = 0{\cdot}0271$).

Conclusion The *p22 phox* C242T polymorphism is not associated with the occurrence or severity of CAD or with a history of MI in Australian Caucasian patients aged ≤ 65 years. However, the polymorphism could be associated with an increased CAD risk in young patients, which requires confirmation in large populations.

Keywords coronary artery disease, ethnic difference, NADH/NADPH *p22 phox* polymorphism.

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Introduction

The NADH/NADPH oxidase system contributes to the generation of the superoxide anion ($\cdot O_2^-$) in endothelial and vascular smooth muscle cells (VSMC) and in phagocytes [1–3]. *p22 phox* is the α-subunit of the cytochrome b_{558} which functions as the final electron transporter from NADPH to molecular oxygen in the system [4]. Oxidative stress in the vasculature induced by $\cdot O_2^-$ via the NADH/NADPH pathway has been implicated in the pathogenesis of hypertension and atherosclerosis [5,6]. Inoue *et al*. recently reported a significant association between the *p22 phox* C242T polymorphism and reduced CAD risk in Japanese subjects [7].

It is established that the *p22 phox* of the NADH/NADPH oxidase system is expressed in human endothelial cells and VSMC and that inhibition of *p22 phox* expression reduces $\cdot O_2^-$ production in VSMC [3,4,8]. The C242T polymorphism is in exon 4 of the *p22 phox* gene and results in the amino acid substitution of His72→Try, at −72, the potential heme-binding site [7]. It could have a direct functional effect on enzymatic activity and $\cdot O_2^-$ production, and influence coronary risk. To explore the possible role of this novel genetic marker for CAD in a Caucasian population, we assessed the frequency of the *p22 phox* C242T polymorphism in a large group of Australian

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patients undergoing coronary angiography. We compared the genotype distributions of this marker in patients with and without angiographically defined CAD, and in patients with different numbers of significantly stenosed (≥ 50% luminal obstruction) major epicardial coronary arteries as well as in those with and without a history of MI.

Methods

Subjects

We studied 689 Australian Caucasians (505 males and 184 females) with and without angiographically defined CAD, who were consecutively referred to the Eastern Heart Clinic at Prince of Wales Hospital for coronary angiography. The severity of CAD was determined by the number of significantly stenosed coronary arteries. Each angiogram was classified as revealing either normal coronary arteries or having no coronary lesion with ≥ 50% luminal stenosis or as having one, two, or three major epicardial coronary arteries with more than 50% luminal obstructions. An angiographically normal coronary artery was defined as a smooth contour and no luminal stenosis. A written consent was obtained from every patient and the study was approved by the Ethics Committee of the University of New South Wales.

We obtained each patient's medical history using a questionnaire with standardised choices of answers to be ticked during the interview and DNA samples were collected for each patient as described previously [9].

Determination of the *p22 phox* C242T polymorphism

PCR-RFLP was used to determine the C242T polymorphism at exon 4 of the NADH/NADPH *p22 phox* gene with the primers described by Inoue *et al*. [7]. The genotypes were identified as CC, TC and TT by SDS-PAGE. The C→T change creates a *Rsa* I recognition site which digests the 348 bp fragment into 188 and 160 bp fragments.

Statistical analysis

All the continuous variables are presented as mean ± SEM. Hardy–Weinberg equilibrium for the *p22 phox* genotype distribution was assessed by a chi-square analysis as described previously [9]. A contingency table chi-square analysis was employed to estimate the contribution of the *p22 phox* polymorphism to the occurrence and severity of CAD, and to evaluate relationships between the *p22 phox* genotypes and other medical conditions including MI, family history of CAD, diabetes mellitus and hypertension. Logistic regression analysis (stepwise linear model) was used to assess the association between the polymorphism and CAD with other known risk factors under control.

Table 1 Risk profiles (mean ± SEM or percentages) in male and female patients

	Men	Women	*P*
n	505	184	
Age (yrs)	55·9 ± 0·3	57·6 ± 0·5	0·008
BMI (kg/m^2)	28·1 ± 0·2	28·0 ± 0·4	0·903
Waist/Hip	0·98 ± 0·01	0·86 ± 0·01	0·0001
TC (mmol L^{-1})	5·3 ± 0·01	5·6 ± 0·01	0·01
Triglyceride (mmol L^{-1})	2·0 ± 0·01	1·7 ± 0·01	0·001
HDL-C (mmol L^{-1})	0·99 ± 0·01	1·27 ± 0·01	0·0001
LDL-C (mmol L^{-1})	3·4 ± 0·03	3·5 ± 0·07	0·238
TC/HDL-C	5·7 ± 0·07	4·7 ± 0·11	0·0001
Apo AI (g L^{-1})	0·95 ± 0·01	1·12 ± 0·02	0·0001
Apo B (g L^{-1})	0·95 ± 0·01	0·92 ± 0·02	0·163
Apo B /AI	1·11 ± 0·02	0·92 ± 0·03	0·0001
Lp(a) (mg L^{-1})	287 ± 13	340 ± 25	0·051
Smoking dose (packyrs)	27·4 ± 1·3	13·3 ± 1·6	0·0001
% Smokers*	74·5%	46·8%	0·0001
% Angiographic CAD	85·4%	61·7%	0·0001
% History of myocardial infarction	41·2%	36·5%	0·260
% Angina	71·5%	79·5%	0·001
% Diabetes	10·9%	14·8%	0·158
% Hypertension	41·3%	52·4%	0·008
% Lipid lowering drug users	28·0%	27·0%	0·794
% β-blocker users	38·7%	36·2%	0·550

P values were obtained by student *t*-test for quantitative variables or chi-squared test categorical variables.

*Smokers were defined as both current and past smokers.

Results

Patients in this study were a high-risk population with suspected diagnosis of CAD. The risk profiles in men and women, and in the patients with or without angiographically demonstrable CAD, were listed in Table 1 and 2. The frequencies of the *p22 phox* C242T genotypes among the 689 patients for CC, TC and TT were 43·3, 45·5 and 11·2% (42·7, 45·3 and 12·0% for men, 43·6, 45·6 and 10·8% for women) respectively. The rare 'T' allele frequency was 0·33 in total population. The genotype distribution was in Hardy–Weinberg equilibrium ($\chi^2 = 0{\cdot}130$, $P > 0{\cdot}05$) and was not different between men and women ($\chi^2 = 0{\cdot}348$, $P = 0{\cdot}840$). Levels of total cholesterol (TC), HDL-C, LDL-C, apo AI, apo B, apo B/AI ratio, Lp (a), TC/HDL ratio, BMI, waist/hip ratio, and life time smoking dose among the genotypes were compared with one-way ANOVA and were not different. The plasma triglyceride levels were not different among the genotypes in males, but in females the triglyceride levels in the mutant TT homozygotes were higher compared to TC and CC genotypes ($P = 0{\cdot}035$). There was no difference in age distributions among patients with different genotypes.

The frequencies for the *p22 phox* CC, TC and TT genotypes and the allele frequencies in patients with and without angiographically defined CAD are shown in Table 3. There were no significant associations between the *p22 phox* genotypes and the occurrence of CAD ($\chi^2 = 0{\cdot}794$, df = 2, $P = 0{\cdot}672$ in men; $\chi^2 = 0{\cdot}442$, df = 2, $P = 0{\cdot}802$ in women). As shown in Table 4, there was no consistent relationship between the frequency of the TT homozygotes or of the 'T' allele frequency and the number of significantly stenosed vessels ($\chi^2 = 4{\cdot}466$, df = 6, $P = 0{\cdot}614$ in men; $\chi^2 = 4{\cdot}736$, df = 6, $P = 0{\cdot}578$ in women). Nor was the polymorphism associated with a history of MI ($\chi^2 = 2{\cdot}310$, $P = 0{\cdot}315$ in men; $\chi^2 = 1{\cdot}178$, $P = 0{\cdot}555$ in women). The *p22 phox* genotypes were also not associated with a family history of CAD ($P = 0{\cdot}419$ in men; $P = 0{\cdot}060$ in women), hypertension ($P = 0{\cdot}463$; $P = 0{\cdot}299$) or diabetes ($P = 0{\cdot}904$; $P = 0{\cdot}902$).

Since the genetically related CAD risk is more likely to start effects from an early age, we also analysed a subset of young patients aged ≤ 45 years ($40{\cdot}8 \pm 0{\cdot}7$ years, $n = 44$ for men and $n = 10$ for women). The number of female patients in this subgroup was too few to have sufficient statistical power for calculations so we only conducted analysis in male patients. There were significant associations between the polymorphism and both the occurrence (Table 5) and severity (Table 6) of CAD in this young male patient subgroup. If a recessive effect was assumed as reported by Inoue and colleagues [7], the prevalence of TC + TT was much higher among patients with CAD (67%) than those without CAD (37·5%). The higher CAD risk associated with TC + TT genotypes was also confirmed in a logistic regression analysis in which other known risk factors were controlled for (odds ratio: 5·71,

Table 2 Risk profiles (mean ± SEM or percentages) in 689 patients with or without angiographically demonstrable coronary stenosis

	CAD (Yes)	CAD (No)	*P*
n	550	139	
Age (yrs)	56·9 ± 0·3	54·5 ± 0·7	0·002
BMI (kg/m^2)	28·2 ± 0·2	27·6 ± 0·4	0·155
Waist/Hip	0·96 ± 0·01	0·91 ± 0·01	0·0001
TC (mmol L–1)	5·4 ± 0·04	5·3 ± 0·08	0·072
Triglyceride (mmol L–1)	2·0 ± 0·04	1·8 ± 0·09	0·054
HDL-C (mmol L–1)	1·04 ± 0·01	1·16 ± 0·02	0·0001
LDL-C (mmol L–1)	3·5 ± 0·04	3·3 ± 0·06	0·006
TC/HDL-C	5·6 ± 0·07	4·9 ± 0·13	0·0001
Apo AI (g L^{-1})	0·98 ± 0·01	1·06 ± 0·03	0·034
Apo B (g L^{-1})	0·96 ± 0·01	0·87 ± 0·02	0·0001
Apo B /AI	1·10 ± 0·02	0·93 ± 0·03	0·0001
Lp(a) (mg L^{-1})	314 ± 13	245 ± 25	0·011
Smoking dose (packyrs)	25·9 ± 1·2	15·2 ± 1·9	0·0001
% Smokers*	71·2%	52·6%	0·0001
Male/Female	440/110	65/74	0·0001
% History of myocardial infarction	45·5%	18·3%	0·0001
% Angina	75·2%	66·7%	0·019
% Diabetes	13·6%	5·9%	0·014
% Hypertension	46·3%	35·8%	0·026
% Lipid lowering drug users	31·1%	15·1%	0·0001
% β-blocker users	38·9%	35·4%	0·493

CAD (Yes) were the patients with angiographically demonstrable coronary lesions and CAD (No) were the patients with angiographically normal coronary arteries. *P* values were obtained by student *t*-test for quantitative variables or chi-squared test categorical variables.

*Smokers were defined as both current and past smokers.

Table 3 The distribution of the *p22 phox* C242T polymorphism in patients with and without angiographically defined CAD

	Male patients		Female patients	
p22 phox genotypes	CAD* (Yes)	CAD (No)	CAD† (Yes)	CAD (No)
TT	52 (11·9%)	7 (10·1%)	13 (11·4%)	6 (8·6%)
TC	200 (45·9%)	29 (42·0%)	50 (43·9%)	33 (47·1%)
CC	184 (42·2%)	33 (47·9%)	51 (44·7%)	31 (44·3%)
Alleles				
T	0·35	0·31	0·33	0·32
C	0·65	0·69	0·67	0·68

CAD (Yes) were the patients with angiographically demonstrable coronary lesions and CAD (No) were the patients with angiographically normal coronary arteries. The frequency distribution of the genotypes among patients with CAD was not different from those without CAD.
*$\chi^2 = 0{\cdot}794$, df = 2, $P = 0{\cdot}672$ in males.
† $\chi^2 = 0{\cdot}442$, df = 2, $P = 0{\cdot}802$ in females.

Table 4 Relationships between the *p22 phox* C242T polymorphism and the severity of CAD in male and female patients

		Number of significantly (≥ 50%) stenosed vessels				
P22 phox genotypes		0	1	2	3	Total
Males*	TT	7 (6·4%)	18 (12·4%)	16 (14·2)	18 (13·0%)	59 (11·7%)
	TC	50 (45·9%)	67 (46·2%)	48 (42·9%)	64 (46·0%)	226 (45·3%)
	CC	52 (47·7%)	60 (41·4%)	48 (42·9%)	57 (41·0%)	217 (43·0%)
	Total	109	145	112	139	505
Females†	TT	7 (7·8%)	6 (15·3%)	2 (6·5%)	4 (16·7%)	19 (10·3%)
	TC	43 (47·8%)	15 (38·5%)	17 (54·8%)	9 (37·5%)	84 (45·7%)
	CC	40 (44·4%)	18 (46·2%)	12 (38·7%)	11 (45·8%)	81 (44·0%)
	Total	90	39	31	24	184

The frequency distribution of the genotypes was not different among patients with different numbers of significantly stenosed vessels.
*$\chi^2 = 4{\cdot}466$, df = 6, $P = 0{\cdot}614$ in males.
† $\chi^2 = 4{\cdot}736$, df = 6, $P = 0{\cdot}578$ in females.

95% CI: 1·22–26·75, $P = 0{\cdot}0271$). Among these 44 young male patients 18 had past history of MI, 31 had angina, 2 had diabetes, 13 had hypertension, 16 were current smokers and 19 were ex-smokers. Their lipoprotein profiles were not different from those of the total patient population.

Table 5 The distribution of the *p22 phox* C242T polymorphism in male patients aged ≤ 45 years with and without angiographically defined CAD

p22 phox genotypes	CAD* (Yes)	CAD (No)
TT	1 (3·6%)	3 (18·8%)
TC	18 (64·3%)	3 (18·8%)
CC	9 (32·1%)	10 (62·5%)
Total	28	16

CAD (Yes) patients were those with angiographically demonstrable coronary lesions and CAD (No) patients were those with angiographically normal coronary arteries. The frequency distribution of the genotypes among patients with CAD was significantly different from those without CAD.
*$\chi^2 = 9{\cdot}672$, df = 2, $P = 0{\cdot}008$.

Discussion

Our study shows that the *p22 phox* C242T polymorphism is not associated with the occurrence or the severity of CAD in all Australian men and women aged ≤ 65 years. However, a significant association between the polymorphism and CAD was observed among a small number of young male patients (≤ 45 years). It should be noted that our analysis among all 689 patients has sufficient statistical power (97·8%) to conclude that there is no association between the polymorphism and CAD. However, the significant relationship between the polymorphism and CAD among young male patients was based on a small number of patients ($n = 44$) with a less satisfactory statistical power (53·4%). This result, therefore, should be interpreted with caution and further studies in larger populations are needed to confirm the finding.

We have also established that there is a considerable difference in the frequency of the rare allele in the Caucasian and Japanese populations. The 'T' allele frequencies in our patients with and without CAD were 0·35 vs. 0·31 for men and 0·32 vs. 0·31 for women whereas they were 0·08 and 0·13 in the 201 Japanese CAD patients and the 201

Table 6 Relationships between the *p22 phox* C242T polymorphism and the severity of CAD in male patients aged ≤ 45 years

	Number of significantly (≥ 50%) stenosed vessels			
p22 phox genotypes	0*	1	2	3
TT	3 (17·6%)	1 (6·3%)	0	0
TC	3 (17·6%)	10 (62·5%)	3 (60·0%)	5 (83·3%)
CC	11 (64·7%)	5 (31·3%)	2 (40·0%)	1 (16·7%)
Total	17	16	5	6

The frequency distribution of the genotypes among patients with different numbers of significantly stenosed vessels.
*$\chi^2 = 13{\cdot}054$, df = 6, $P = 0{\cdot}042$.

controls [7]. Our results were further different from those of Inoue *et al*. [7] in that for their patients the rare 'T' allele was associated with reduced CAD risk, whereas in our patients the rare TT homozygotes or 'T' allele tended to be more prevalent in patients with CAD, and in patients with ≥ 1 significantly stenosed coronary arteries (Table 3, 4). This was supported statistically by the findings in a subset of young men (≤ 45 years), among whom TC + TT genotypes had an increased CAD risk than that of the common CC genotype (odds ratio: 5·71, 95% CI: 1·22–26·75).

In conclusion, the NADH/NADPH *p22 phox* C242T polymorphism is common in Australian Caucasians with an allele frequency of 0·33, which is more than three times the frequency recorded in the Japanese population. The polymorphism was not associated with the occurrence or severity of angiographically defined CAD or with a history of MI in patients aged ≤ 65 years. However, the polymorphism could be associated with an increased CAD risk among younger patients, e.g. ≤ 45 years. Our findings are opposite to those found in the Japanese population among whom a reduced CAD risk was associated with the polymorphism. Further studies among larger populations are needed.

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