

The *StuI* Polymorphism on Exon 8 of the Low Density Lipoprotein (LDL) Receptor Gene: Prevalence and Impact on Serum Lipid Levels in an Asian Cohort

M L Choong,**BSc (Hons), M Med Sc*, S K Sethi,***MBBS, PhD, MRCPath*, E S C Koay,****PhD, MRCPath, FAACB*

Abstract

A single nucleotide (A→G) substitution in codon 370 (exon 8) of the low density lipoprotein (LDL) receptor gene results in loss of a *StuI* restriction site and an amino acid change (Ala₃₇₀→Thr) in the translated protein. This biallelic polymorphism has been associated with significant variations in plasma lipid concentrations in several Caucasian populations. We investigated its prevalence and impact on lipid metabolism in 539 Singaporeans of Chinese, Malay and Indian descent. The average frequency of 0.003 for the Thr370 allele and heterozygosity of 0.0056 were 10- to 20-fold lower than those reported in Caucasians (range 0.075 to 0.107). Distribution of the genotypes satisfied the Hardy-Weinberg equilibrium ($\chi^2=0.004$, $P=0.948$). We also found ethnic variation in the allele frequencies of Thr370 among the three races studied (0.009 in Indians, 0.007 in Malays, and 0.003 in Chinese). However, this needs to be confirmed with larger numbers. No significant correlation between genotype and serum concentrations of total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol, LDL-cholesterol, lipoprotein(a), apolipoprotein A and apolipoprotein B was found in our study. This argues strongly against a major contribution or impact of this intragenic polymorphic locus on modulating the serum lipid profiles of the study subjects.

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Introduction

The low density lipoprotein (LDL) particles in the plasma carry most of the cholesterol in circulation. High levels of plasma cholesterol and LDL-cholesterol are associated with increased risk of coronary artery disease (CAD).¹⁻³ LDL is removed by both non-receptor-mediated and receptor-mediated pathways. The LDL receptor recognises and binds to a specific region of apolipoprotein (apo) B-100, the major constituent protein of LDL, followed by endocytosis of the receptor-ligand complex. Over 150 mutations in the LDL receptor gene sequence have been reported⁴ as well as a large number of polymorphisms.⁵⁻⁷ Different mutations and polymorphisms in the LDL receptor gene affect the function of the protein in different ways and to different extents.^{6,8-12} The rare and highly deleterious mutations causing familial hypercholesterolaemia (FH) result in amino acid substitutions in the strategic receptor binding site of the LDL receptor and have been associated with gross hypercholesterolaemia and premature atherosclerosis.⁸⁻¹¹ In contrast, the alanine (Ala)

to threonine (Thr) change at codon 370 caused by a single nucleotide (A→G) substitution in exon 8,¹³ and resulting in loss of a restriction site for the *StuI* endonuclease,¹⁴ appears to have a smaller effect on the function of the receptor and does not result in such a drastic modulation of plasma LDL-cholesterol concentration.¹² This is the only amino acid substitution in the LDL receptor described to date that is not associated with FH. However, the polymorphism is quite common among Europeans and other Caucasian populations (allele frequency ranges from 0.04 to 0.08) and thus carries a sizeable contribution in determining lipid levels in such populations.^{5,6,12,13} The wild-type amino acid residue is alanine in man and rabbit, and valine in mouse, hamster and rat.¹² These two non-polar amino acids are structurally similar, suggesting that a change to the polar threonine could alter the function of the receptor and affect the rate of LDL uptake from the circulation. We investigated the effect of this polymorphism on blood lipid levels in healthy representatives of three Asian races living in close proximity in Singapore. No population-based studies

* Research Assistant and PhD Candidate

** Senior Lecturer and Consultant

*** Senior Lecturer

Department of Pathology

National University of Singapore

Address for Reprints: Dr E S C Koay, Department of Pathology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260.

E-mail: patkoaye@nus.edu.sg

have yet been undertaken in East Asians to determine the variability of such effects that may be attributable to race.

Materials and Methods

Study Subjects

A total of 539 unrelated individuals (age range 17 to 83 years) attending an outpatient, multiphasic health screening outpatient clinic at the National University Hospital, Singapore, were recruited for this study. These were adults who were having pre-employment or insurance policy health screens or annual medical check-ups, and were essentially healthy individuals. They represented consecutive registrations at the clinic over a 60-week period, after individuals with clinical evidence or family history of FH were excluded. This sampling design is to avoid bias in sampling and to obtain a cross-section of healthy subjects with a good ethnic mix. There were 306 Chinese (149 males:157 females), 125 Malays (58 males:67 females) and 108 Indians (55 males:53 females). Blood samples were collected after an overnight fast for the lipid profile assays and for genotyping.

Lipid, Lipoprotein and Apolipoprotein Assays

Serum total cholesterol, triglyceride, high density lipoprotein (HDL)-cholesterol, apolipoprotein (apo) A-I, apo B and lipoprotein(a) [Lp(a)] were measured using the methods listed below: total cholesterol and triglycerides (Johnson and Johnson dry chemistry slides ran on the Vitros 750 autoanalyser), HDL-cholesterol (Boehringer Mannheim dry chemistry strips, using the Reflotron analyser), apo A-I and apo B (immunonephelometric assays on the Beckman Array Protein Analyser), and Lp(a) (enzyme immunoassay from Perimmune Diagnostics, USA). LDL-cholesterol was estimated by using the Friedewald calculation, based on the measured values of total cholesterol, triglycerides and HDL-cholesterol from the same individual.¹⁵

DNA Extraction and Polymerase Chain Reaction (PCR)

DNA was extracted from peripheral leukocytes (EDTA-anticoagulated blood) by ethanol precipitation after removal of cellular proteins using a salting-out procedure described by Miller et al.¹⁶ A 196 bp fragment of exon 8 of the LDL receptor gene was amplified by PCR and digested with *Stu*I restriction enzyme as described by Gudnason et al,¹² with slight modification. We used a 60°C annealing temperature instead of 57°C to reduce non-specific products. The restriction fragment length polymorphisms (RFLPs) were analysed by electrophoretic separation using a 10% polyacrylamide gel and ethidium bromide staining.

Statistical Analyses

Calculations of allele frequencies, heterozygosities and polymorphism information content (PIC), and testing

for Hardy-Weinberg equilibrium were all performed using the LINKAGE programmes.¹⁷ As ANOVA is a parametric test which assumes normality of data and homogeneity of variance, all the lipid traits were pre-tested with Kolmogorov-Smirnov's test of normality and Levene's test for homogeneity of data distributions before applying the ANOVA (the null hypothesis of homogeneity and normality were accepted when $P > 0.05$). In addition, skewed distributions, e.g. those of serum triglyceride and Lp(a), were normalised by natural logarithmic transformation prior to performing ANOVA statistics. Simple factorial ANOVA was performed to determine the significance of differences for lipid traits between genotypes with age as covariate. Significance levels were set at 0.05 for all cases. The software SPSS for Windows version 6.1 was used to perform all statistical calculations described in this section.

Results

Of the 539 subjects studied, 536 of them (99.44%) were homozygous for the Ala/Ala allele at the *Stu*I polymorphic site. There were only three Ala/Thr heterozygotes and no Thr/Thr homozygotes in the cohort studied. Individuals with the Ala/Ala and Ala/Thr genotypes can be clearly distinguished by their *Stu*I RFLPs on a 10% polyacrylamide gel, as shown in Figure 1. The total genotype frequency did not deviate from the Hardy-Weinberg equilibrium ($P = 0.948$). The calculated estimated heterozygosity (maximum likelihood) and PIC are shown in Table I. The overall Ala370 allele fre-

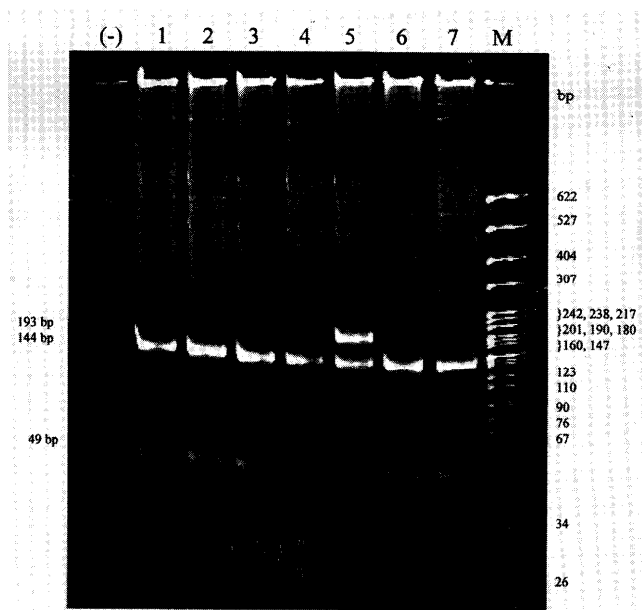


Fig. 1. Restriction fragment length polymorphisms (RFLPs) of individuals with Ala/Ala and Ala/Thr genotypes for the *Stu*I restriction site on exon 8 of the LDL receptor gene. The RFLPs were run on a 10% polyacrylamide gel and stained with ethidium bromide. Lane (-): Negative control; Lanes 1, 2, 3, 4, 6, 7: Homozygotes for the Ala/Ala genotype; Lane 5: Heterozygote for the Ala/Thr genotype; Lane M: DNA size marker (*Msp*I digest of pBR322).

TABLE I: LDL RECEPTOR EXON 8 *StuI* POLYMORPHISM IN THE SINGAPORE POPULATION

		Genotype		Allele		Estimated heterozygosity	PIC
		Ala/Ala	Ala/Thr	Ala370	Thr370		
Chinese	M	149 (100.0%)	0 (0.0%)	298 (100.0%)	0 (0.0%)	0.0033 ± 0.0033 (0.0000 to 0.0096)	0.0033
	F	156 (99.4%)	1 (0.6%)	313 (99.7%)	1 (0.3%)		
Malay	M	58 (100.0%)	0 (0.0%)	116 (100.0%)	0 (0.0%)	0.0080 ± 0.0079 (0.0000 to 0.0235)	0.0079
	F	66 (98.5%)	1 (1.5%)	133 (99.3%)	1 (0.7%)		
Indian	M	54 (98.2%)	1 (1.8%)	109 (99.1%)	1 (0.9%)	0.0093 ± 0.0092 (0.0000 to 0.0272)	0.0092
	F	53 (100.0%)	0 (0.0%)	106 (100.0%)	0 (0.0%)		
Total		536 (99.4%)	3 (0.6%)	1075 (99.7%)	3 (0.3%)	0.0056 ± 0.0032 (0.0000 to 0.0118)	0.0055

Total observed heterozygosity = $\frac{3}{539} = 0.0056$ (95% CI: 0.0012 to 0.0162)

Hardy-Weinberg equilibrium: $\chi^2 = 0.004$, $P = 0.948$

Heterozygosities are reported in mean ± SE (standard error), numbers in parentheses in the heterozygosity column are 95% confidence intervals

LDL: low density lipoprotein; PIC: polymorphism information content

TABLE II: SERUM LIPID PROFILES OF INDIVIDUALS WITH ALA/ALA AND ALA/THR GENOTYPES AT CODON 370 (EXON 8) OF THE LDL RECEPTOR GENE

	Ala/Ala	Ala/Thr
n	536	3
TC (mmol/L)	5.97 (1.27)	5.62 (0.61)
HDL-C (mmol/L)	1.00 (0.32)	1.12 (0.36)
LDL-C (mmol/L)	4.22 (1.17)	4.34 (0.56)
Apo A-I (mg/dL)	134.11 (20.92)	137.95 (20.79)
Apo B (mg/dL)	124.85 (15.00)	125.75 (12.10)
ln(Tg)	0.45 (0.63)	0.01 (0.17)
ln[Lp(a)]	2.11 (1.17)	2.76 (0.65)

Results are in mean values with SD in parentheses

$P > 0.1$ for all lipid parameters between the two genotypes

Apo: apolipoprotein; HDL-C: high density lipoprotein cholesterol;

LDL-C: low density lipoprotein cholesterol; ln: natural logarithm;

Lp(a): lipoprotein(a); TC: total cholesterol; TG: triglycerides

quency, heterozygosity and PIC values in the Singapore population were 0.997, 0.0056 and 0.0055, respectively. Table II shows the results of a simple factorial ANOVA, adjusted for age as covariate, comparing the effect of the genotype Ala/Thr on lipid and lipoprotein levels, using Ala/Ala as reference genotype. There were no significant differences between the two genotypes ($P > 0.1$ in all cases).

Discussion

The small percentage (0.56%) of heterozygotes (Ala/Thr) and the absence of any homozygotes for the rare allele (Thr/Thr) in our study population deviates markedly from the results of previous studies on 7 other population (Table III). Compared to other races from around the world, the three ethnic groups in our study had higher Ala370 allele frequencies (or much lower Thr370 allele frequencies) and lower heterozygosities and PIC values. Data on other Asian population were not available for comparison. The overwhelming preponderance of the Ala/Ala genotype is a consistent

feature for all the three ethnic groups studied, and holds true for both males and females. The results argue strongly against this particular intragenic polymorphism on exon 8 of the LDL receptor gene being a useful or informative DNA marker for identification of individuals with increased risk for hyperlipidaemia and atherosclerosis. It is unclear to us what causes the close to 100% homogeneity in allelic distribution in our Asian subjects, which was not found in Caucasians.^{6,7,12,18-22}

The allele for Thr370 has been reported by two research groups to be associated with raised plasma levels of total cholesterol, LDL-cholesterol and apo B in males.^{7,18} Gudnason et al¹² found that men with the Thr370 allele were in the upper 75th percentile of the cholesterol distribution for the appropriate group, even after adjusting for the apo E genotype, which can be translated to mean that statistically they could have a 20% greater risk for coronary artery disease.* In several other studies, a decrease in the frequency of the Ala370 allele was demonstrated in FH patients: 0.942 in the Swiss,¹⁹ 0.899 in the South Africans,²⁰ and 0.93 (compared to 0.99 in the control group) in the Czechs.²¹ However, all the FH patients in a German population were found to be homozygous for the Ala370 allele."

The lack of any significant differences in the serum lipid profiles of individuals with Ala/Ala ($n = 536$) and Ala/Thr ($n = 3$) in our study appears to be in contrast to the above reports. However, the very low heterozygosity of this polymorphism (0.0056) in our subjects precludes any meaningful correlations between genotypes with serum lipid and lipoprotein concentrations. Our data on the association of Thr370 and hyperlipidaemia, at best, suggest a lack of association but the role of allele Thr370 in the process or outcome of atherosclerosis remains unclear. Interestingly, the trend in ethnic variations in the allele frequencies of Thr370 (Indians>Malays>Chinese) is similar to that of incidence of coronary heart disease in the same three ethnic

TABLE III: COMPARISONS OF ALLELE FREQUENCY, HETEROZYGOSITY AND PIC AMONG DIFFERENT ETHNIC GROUPS

Ethnic group	Ala370 frequency	Heterozygosity	PIC	Reference
Chinese ^a	0.998	0.0033	0.0033	This study
Malay ^b	0.996	0.0080	0.0079	This study
Indian ^c	0.995	0.0093	0.0092	This study
Singaporean*	0.997	0.0056	0.0055	This study
Icelandic	0.950	0.0900	0.0860	Gudnason et al 1995, ¹²
Israelite	0.960	0.0768	0.0739	Friedlander et al 1995, ²²
Briton	0.970	0.0582	0.0565	Humphries et al 1993, ⁷
Swiss	0.961	0.0750	0.0730	Miserez et al 1993, ¹⁹
German	0.899	0.1820	0.1650	Miserez et al 1993, ¹⁹
North American	0.943	0.1070	0.1020	Leitersdorf et al 1989, ⁷
South African	0.946	0.1020	0.0970	Koltze et al 1989, ²⁰

* Average of Singaporeans of Chinese^a, Malay^b and Indian^c descent

PIC: polymorphism information content

groups.²³ Further studies involving larger numbers of subjects from each of these groups may provide better definition of any association.

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