

Relation of C-Reactive Protein and Carotid Intima Media Thickness in Taiwanese With Familial Hypercholesterolemia

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The aim of this study was to investigate the potential relationships between the carotid intima media thickness (carotid IMT), high sensitive C-reactive protein (hsCRP), and cholesterol burden in heterozygous familial hypercholesterolemia (FH) subjects. Thirty-two genetically-verified heterozygous patients with FH and 34 healthy controls were recruited into our study in Taiwan. We measured conventional risk factors, hsCRP, and carotid IMT of study subjects. The cholesterol-year score was used to estimate the lifetime cholesterol burden. Subjects with heterozygous FH had significantly elevated total cholesterol, elevated low-density lipoprotein cholesterol, and increased carotid IMT compared with control subjects. Carotid IMT correlated well with the cholesterol-year score. In patients with FH, univariate analysis showed that hsCRP was highly correlated with carotid IMT. Multiple linear regression analysis indicated that hsCRP was the only independent predictor of carotid IMT in patients with FH. In conclusion, patients with heterozygous FH had significantly higher carotid IMT and the level of hsCRP was independently associated with atherosclerotic progression. (R: 0.639, R²: 0.408, p <0.001). © 2008 Elsevier Inc. All rights reserved. (Am J Cardiol 2008;102:184–187)

Studies have shown the correlation between inflammation markers and subclinical atherosclerosis markers, including coronary artery calcium and arterial stiffness in subjects with familial hypercholesterolemia (FH).^{1,2} However, the correlation of high sensitive C-reactive protein (hsCRP) and carotid intima media thickness has not been shown so far in FH subjects. In the current study, we recruited FH subjects who had no major cardiovascular risk factors except hypercholesterolemia. Our primary goal was to investigate potential relationships between the carotid intima media thickness (carotid IMT), the hsCRP level, and the cholesterol burden in these subjects.

Methods

From October 2004 to November 2006, patients with low-density lipoprotein cholesterol (LDL-C) >190 mg/dl and positive family history of hypercholesterolemia were enrolled in a genetic screening program for familial hypercholesterolemia in Taiwan. Other secondary causes of hypercholesterolemia were excluded, including nephrotic syndrome,

liver disease, hypothyroidism, and diabetes mellitus. Methodology used for mutation detection was described previously.³ Family members who were genetically verified as not carrying an FH-related mutation allele and with LDL-C <130 mg/dl were enrolled as the control group. The protocol was approved by the institutional review board of the hospital, and informed consent was obtained from each patient.

All medications that could affect lipid levels were discontinued 4 weeks previous to the study. After an overnight fast of 12 to 14 hours, serum levels of total plasma cholesterol, high-density lipoprotein-cholesterol, and triglycerides were measured with commercially-available kits (Roche, Mannheim, Germany). LDL-C was calculated according to the formula of Friedewald et al.⁴ The concentrations of apolipoprotein A1 and apolipoprotein B were measured by nephelometry (Behring Diagnostic, Marburg, Germany). A highly sensitive latex-based immunoassay (Dade Behring, Milton Keynes, United Kingdom) was used to determine levels of CRP. The cholesterol-year score was used to quantify the cholesterol burden. It was calculated with the equation of Hoeg, et al⁵: cholesterol year score = yearly mean cholesterol serum level × age. In the present study, the serum cholesterol levels measured during clinic visits were averaged for the calculation.

The protocol for measuring carotid IMT consisted of scanning the carotid arteries longitudinally in the 3 segments covering the carotid bulb, and the lateral extent of each segment was used to estimate media thickness. A 7.5-MHz probe with fine transducer angulations was used for clearly displaying both the blood-intima and media-adventitia boundaries on both the near and far walls of the artery. The focus was positioned at a 40-mm depth within the near or the far wall, depending on the desired optimized

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Table 1
Characteristics of controls and individuals with familial hypercholesterolemia

Variable	Control (n = 34)	Familial Hypercholesterol Subjects (n = 32)
Age (yrs)	42.1 ± 17.0	36.0 ± 17.8
Men/women	17/17	18/14
Body weight (kg)	58.3 ± 15.2	59.6 ± 17.6
Body mass index (kg/m ²)	23.4 ± 3.9	22.1 ± 4.9
Waist circumference (cm)	78.3 ± 8.5	78.6 ± 11.6
Systolic blood pressure (mm Hg)	110 ± 11	116 ± 14
Diastolic blood pressure (mm Hg)	65 ± 10	67 ± 11
Fasting blood glucose (mg/dl)	101 ± 31	93 ± 8
Total cholesterol (mg/dl)	197 ± 38	300 ± 74 [†]
Triglyceride (mg/dl)	133 ± 65	133 ± 76
Low-density lipoprotein cholesterol (mg/dl)	120 ± 38	218 ± 66 [†]
High-density lipoprotein cholesterol (mg/dl)	51 ± 18	57 ± 12
Total cholesterol/high-density lipoprotein cholesterol	4.1 ± 0.9	5.5 ± 1.5 [†]
Cholesterol-year score (mg-y/dl)	8,277 ± 3,391	11,077 ± 6,235*
Apolipoprotein B (mg/dl)	99 ± 30	152 ± 41 [†]
Apolipoprotein A1 (mg/dl)	132 ± 20	133 ± 31
High sensitive C-reactive protein (mg/dl)	0.11 ± 0.14	0.12 ± 0.17
Carotid intima media thickness (cm)	0.07 ± 0.01	0.11 ± 0.09*

Values are means ± SDs or numbers of patients (percentages).

* p < 0.05 versus control subjects.

[†] p < 0.001 versus control subjects.

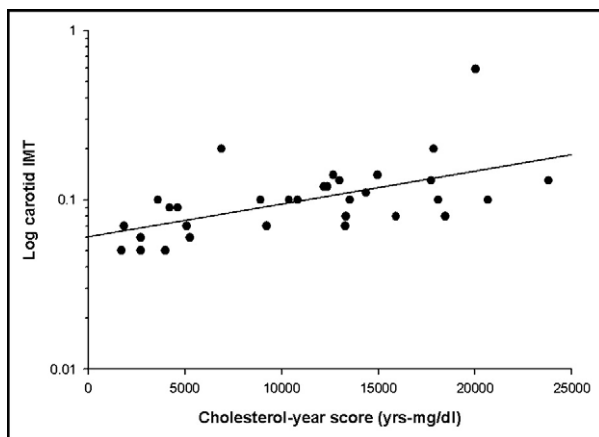


Figure 1. Semi-log scatter plot of the log carotid intima medial thickness (y axis) and cholesterol-year score (x axis). The solid line shows the correlation between the carotid IMT and the cholesterol-year score ($r = 0.602$, $p < 0.001$). IMT = intima media thickness.

image during scanning. IMT was measured outside the plaque lesion in cases where plaques were present. At least 4 IMT sites were isolated and measured for each segment (12 sites for each patient). All IMT measurements were performed offline with image-analysis software. Image sequences were recorded and subsequently reviewed frame by frame to select the best-quality images for measurement. Leading edges were traced with calipers for measurement of the far walls, and trailing edges were traced for measure-

Table 2
Spearman's correlation coefficients for risk factors in 35 heterozygous familial hypercholesterolemia subjects

Variable	Carotid Intima Media Thickness		High Sensitive C-Reactive Protein	
	r	p Value	r	p Value
Age	0.623	<0.001	0.587	<0.001
Sex	-0.027	NS	-0.076	NS
Body mass index	0.636	<0.001	0.672	<0.001
Waist circumference	0.638	<0.001	0.707	<0.001
Systolic blood pressure	0.400	0.023	0.540	0.001
Diastolic blood pressure	0.412	0.019	0.580	0.001
Total cholesterol	0.282	NS	0.205	NS
High-density lipoprotein	-0.109	NS	-0.116	NS
Low-density lipoprotein	0.284	NS	0.310	NS
Triglyceride	0.505	0.002	0.000	0.002
Apolipoprotein B	0.367	0.039	0.547	0.001
Apolipoprotein A1	0.056	NS	0.075	NS
Fasting blood glucose	0.417	0.018	0.114	NS
Cholesterol-year score	0.602	<0.001	0.574	0.001
High sensitive C-reactive protein	0.500	0.004	—	—

NS indicates not significant.

ment of the near walls. IMT was calculated as a composite measure (the mean of 12 sites).

Results are expressed as means and SDs for continuous variables and proportions for categorical variables. Between-group comparisons were performed using the Wilcoxon 2-sample test and the one-way analysis of variance test (ANOVA) where appropriate. To obtain a normal distribution, logarithmically transformed values for carotid IMT were used in the statistical analysis. In FH subjects, Spearman's correlation coefficients were determined to assess the association between continuous carotid IMT, plasma lipids and lipoproteins, blood pressure values, and hsCRP levels. To test whether these variables had independent effects on the carotid IMT of FH subjects, a stepwise multiple linear regression analysis was performed and the predictors for logarithmic carotid IMT were determined. All tests were 2-sided and a value of $p < 0.05$ was considered statistically significant.

Results

Thirty-two subjects with genetically-verified mutations in the LDL receptor gene and 34 healthy family members with genetically-verified wild-type FH-related genes were enrolled in this study. Screening by inclusion criteria ensured that none of the participants had a history of cardiovascular disease, hypertension, or diabetes mellitus. Three of the FH subjects and one of the control subjects were current smokers. **Table 1** lists the baseline characteristics of the study subjects. Compared with the control group, FH subjects had significantly elevated serum levels of total cholesterol, LDL cholesterol, and apolipoprotein B. In FH subjects, both the calculated cholesterol-year score, a marker of total cholesterol burden, and carotid IMT, which is a marker of systemic atherosclerosis, were significantly higher than those of the control subjects. Furthermore, as shown in **Figure 1**,

Table 3
Basic characteristics in subjects with familial hypercholesterolemia grouped by carotid intima media thickness

Variable	Carotid intima media thickness ≤ 1 mm (n = 21)	Carotid intima media thickness > 1 mm (n = 11)	p Value
Age (yrs)	28.8 $\pm$ 15.7	49.6 $\pm$ 14.8	0.001
Men/women	12/9	6/5	0.888
Systolic blood pressure (mm Hg)	112 $\pm$ 12	123 $\pm$ 17	0.056
Diastolic blood pressure (mm Hg)	65 $\pm$ 10	73 $\pm$ 11	0.045
Body mass index (kg/m ²)	20.6 $\pm$ 4.9	24.9 $\pm$ 3.3	0.014
Waist circumference (cm)	74.9 $\pm$ 12.3	85.7 $\pm$ 5.2	0.010
Fasting blood glucose (mg/dl)	91 $\pm$ 7	97 $\pm$ 8	0.066
Total cholesterol (mg/dl)	294 $\pm$ 78	310 $\pm$ 67	0.577
Triglyceride (mg/dl)	112 $\pm$ 62	173 $\pm$ 87	0.027
Low-density lipoprotein cholesterol (mg/dl)	214 $\pm$ 68	227 $\pm$ 64	0.600
High-density lipoprotein cholesterol (mg/dl)	58 $\pm$ 13	54 $\pm$ 10	0.384
Total cholesterol/ high-density lipoprotein cholesterol	5.3 $\pm$ 1.6	5.8 $\pm$ 1.1	0.287
Cholesterol-year score (y-mg/dl)	8978 $\pm$ 6028	15084 $\pm$ 4572	0.006
Apolipoprotein B (mg/dl)	143 $\pm$ 42	169 $\pm$ 39	0.099
Apolipoprotein A1 (mg/dl)	132 $\pm$ 33	135 $\pm$ 27	0.806
High sensitivity C-reactive protein (mg/dl)	0.08 $\pm$ 0.09	0.22 $\pm$ 0.24	0.023

Values are means $\pm$ SDs.

the cholesterol-year score correlated well with the carotid IMT ($r = 0.602$, $p < 0.001$) in this study.

As shown in Table 2, the carotid IMT was significantly correlated with age, body mass index, waist circumference, systolic and diastolic blood pressures, triglyceride levels, apolipoprotein B levels, fasting blood glucose, the cholesterol-year score, and hsCRP levels. In addition, the hsCRP level was associated with age, body mass index, waist circumference, triglyceride levels, apolipoprotein B levels, and the cholesterol-year score.

We further divided FH subjects into 2 groups according to the extent of carotid IMT (cut-off value: 1 mm). Listed in Table 3, FH subjects with a carotid IMT > 1 mm were older and had higher diastolic blood pressure, body mass index, waist circumference, and triglyceride levels than did FH subjects with IMT ≤ 1 mm. In addition, the former group also had significantly elevated cholesterol-year scores and hsCRP levels compared with the latter group. The multiple linear stepwise regression analysis adjusted for age, gender, systolic/diastolic blood pressure, body mass index, total cholesterol, LDL-C, and fasting blood glucose level indicated that the hsCRP level was an independent predictor for the extent of carotid IMT (Partial R: 0.639, R^2 : 0.408, $p < 0.001$).

Discussion

The present study demonstrates that aggravated atherosclerosis was present in patients with familial hypercholester-

olemia, even in the absence of other major cardiovascular risk factors. In addition, we demonstrate that both the cholesterol burden and the inflammatory state may contribute to the progression of atherosclerosis.

The carotid intima media thickness is a good surrogate marker for atherosclerosis and is commonly used to measure the extent of atherosclerosis.^{6,7} In addition, low-grade inflammation has been implicated in the progression of atherosclerosis,^{8,9} and hsCRP, a marker of inflammation, has been established as a useful predictor of the progression of early carotid atherosclerosis in patients with mild to moderate cardiovascular risk.¹⁰ Ueland et al¹¹ demonstrated that hsCRP was significantly elevated in children with heterozygous familial hypercholesterolemia, indicating that vascular inflammation was present in the early stages of hypercholesterolemia. In addition, Rodenburg et al¹² reported a correlation between the oxidized phospholipid:Apo B-100 ratio and hsCRP levels among children with heterozygous familial hypercholesterolemia. Our previous studies^{1,2} in FH subjects have also shown that both vascular stiffness and coronary calcium levels were aggravated, and elevated hsCRP was an independent predictor for their progression. However, Lorenz et al¹³ reported that hsCRP was not an independent causal factor for the initiation and progression of early atherosclerotic changes of the carotid arteries. The discrepancy may be the result of a different study population. We recruited relatively homogeneous subjects with genetically verified heterozygous familial hypercholesterolemia and excluded those with other conventional risk factors. Our findings are in good agreement with previous observations in similar study populations.^{11,12}

In the current study, the baseline hsCRP level did not differ significantly between FH and control subjects, which may be the result of a limited study population. Despite a relatively small sample size, the correlations between carotid IMT and hsCRP still exist in this analysis of heterozygous FH subjects.

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