Atherosclerosis

Association of Pathobiologic Determinants of Atherosclerosis in Youth Risk Score and Carotid Artery Intima-Media Thickness in Asymptomatic Young Heterozygous Familial Hypercholesterolemia Patients

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Background: Familial hypercholesterolemia (FH) is an inherited disorder characterized by extremely high cholesterol level, accelerated atherosclerosis and premature cardiovascular disease. We need an early risk stratification method for this population. A risk score formula to estimate the probability of advanced atherosclerosis using coronary heart disease risk factors was developed for persons 15-34 years of age by the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. This study's aim was to investigate the relation between PDAY risk score and carotid intima-media thickness (IMT) and inflammation markers in asymptomatic young FH subjects.
Methods: We included 23 heterozygous FH patients (mean age 27) and 22 healthy control subjects (mean age 33). We computed the PDAY risk scores and measured the high-sensitive C-reactive protein (hsCRP) and carotid IMT.
Results: In FH subjects, univariate analysis showed PDAY risk score was significant correlated with hsCRP and carotid IMT. Comparing FH subjects with the presence of carotid atheroma plaque (IMT > 1 mm) or without the plaque (IMT< 1 mm), we found significantly higher PDAY risk score and hsCRP value in the presence group. By receiver operating characteristic curve, both hsCRP and PDAY risk score could predict the presence of carotid plaque. The multivariate analysis showed the correlation between PDAY risk score and carotid plaque (odds ratio for a 1-unit increase in the risk score was 1.161, 95% confidence interval: 1.027-1.312, p = 0.017).
Conclusion: In young heterozygous FH subjects, PDAY risk score is strongly correlated with carotid IMT, and it might be a simple and useful tool for cardiovascular disease risk stratification in this population.

Key Words: Carotid intima-media thickness • C-reactive protein • Familial hypercholesterolemia • Pathobiologic Determinants of Atherosclerosis in Youth Risk Score

INTRODUCTION

Familial hypercholesterolemia (FH) is a common inherited disorder of lipoprotein metabolism that is characterized by very high levels of serum cholesterol, accelerated onset of atherosclerosis, and premature cardiovascular disease.[1] We have published some studies about the relation of inflammation and atherosclerosis surrogate markers in FH subjects.[2-4] However, to predict the advanced subclinical atherosclerosis, the traditional risk stratification tools might not be adequate due to the early

Received: August 18, 2010 Accepted: March 3, 2011
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onset of atherosclerosis in these subjects. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study of autopsy findings in subjects 15 to 34 years of age developed a risk score using coronary heart disease risk factors (gender, age, serum lipoprotein concentrations, smoking, hypertension, obesity, and hyperglycemia) to estimate the probability of advanced atherosclerotic lesions in the coronary arteries.[5] It also had been proved to predict advanced coronary artery atherosclerosis in middle-age persons. The PDAY risk score provides an inexpensive and simple tool for clinicians to make the risk stratification of coronary heart disease.

In this report, we recruited asymptomatic FH subjects who were younger than 45 years old and showed the correlation between PDAY score, inflammation markers and carotid intima-media thickness. Then we tested the hypothesis that PDAY risk score might predict carotid intima-media thickness independently in these subjects.

METHODS

From October 2004 to November 2006, patients younger than 45 years old with 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. The methodology used for mutation detection has been described previously.[6] 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 four weeks prior to the study. After an overnight fast of 12-14 hours, serum levels of total plasma cholesterol, high-density lipoprotein-cholesterol, and triglycerides were measured with commercially available kits (Roche, Mannheim, Germany). LDL cholesterol was calculated according to the formula of Friedewald et al.[7] 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, UK) was used to determine levels of CRP. The protocol for measuring carotid IMT consisted of scanning the carotid arteries longitudinally in the 3 segments covering the carotid bulb, with the lateral extent of each segment being used to estimate media thickness. A 7.5-MHz probe with fine transducer angulations was used for clearly displaying both the lumen-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 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 wall, and trailing edges were traced for measurement of the near walls. IMT was calculated as a composite measure (the mean of 12 sites). An artery was classified as being affected by atherosclerotic plaque if there was a localized thickening ≥ 1 mm that did not uniformly involve the whole left or right carotid artery with or without flow disturbance.

The PDAY risk score was derived from the associations of cardiovascular disease risk factors measured postmortem with atherosclerotic lesions in the coronary arteries of 1,117 subjects examined at autopsy.[5,8] The target lesions were American Heart Association grade IV/V lesions in the left anterior descending coronary artery, > 9% of the intimal surface area of the right coronary artery involved with gross raised lesions, or both. Risk factors included HDL and non-HDL cholesterol concentrations and thiocyanate concentration (as a marker of smoking) in postmortem serum, BMI at autopsy > 30 kg/m^2 to define obesity hyperglycemia assessed by a red blood cell glycohemoglobin level > 8%, and hypertension assessed by the intima thickness of the small renal arteries. The normalization for the PDAY risk score was such that 1 point was equivalent to 1 year of age. Point values for the risk factors are listed in Table 1.

Table 1. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) risk score for predicting advanced atherosclerosis lesions

Risk factors	PDAY coronary artery risk score
Age (years)	
15-19*	0
20-24	5
25-29	10
30-34	15
Gender	
Male*	0
Female	-1
LDL-Cholesterol (mg/dl)	
< 130*	0
130-159	2
160-189	4
190-219	6
≥ 220	8
HDL-Cholesterol (mg/dl)	
< 40	1
40-59*	0
≥ 60	-1
Smoking	
Nonsmoker*	0
Smoker	1
Blood pressure	
Normotensive	0
Hypertensive	4
Body mass index (kg/m^2)	
Males	
≤ 30*	0
> 30	6
Females	
≤ 30	0
> 30	0
Hyperglycemia/diabetes	
Normoglycemic/nondiabetic*	0
Hyperglycemic/diabetic	5

Adapted from McMahan et al.[5]; Reference category.

All of the results were expressed as means with standard deviation for continuous variables and proportions for categorical variables. Comparisons between groups were performed using the Wilcoxon two-sample test. Spearman's correlation coefficients were determined to assess the association between PDAY risk score, carotid IMT, plasma lipid profile, lipoprotein, blood pressure, and hsCRP level. To compare whether variables had the effect of predicting the presence of carotid atheroma plaque, the area under the receiver operating characteristic (ROC) curve with confidence interval and multivariate logistic regression were performed. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

RESULTS

Twenty-three subjects with genetically-verified mutations in the LDL receptor gene and 22 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. Three of the FH subjects and one of the control subjects were current smokers. In the FH subjects, 8 of 23 had tendon xanthoma (35%). Table 2 presents 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, the carotid IMT, a marker of systemic atherosclerosis, was significantly higher than that of the control subjects. The PDAY risk score of FH subjects was higher than that in the control group, although the p value was borderline significant ($p = 0.072$). Furthermore, as shown in Figures 1A and B, PDAY risk score correlated well with the carotid IMT and hsCRP ($r = 0.558$, $p < 0.001$; $r = 0.430$, $p = 0.003$ by Spearman's correlation coefficients).

We further divided the FH subjects into two groups according to the presence of carotid atheroma plaque or not. As shown in Table 3, the FH subjects with the presence of carotid atheroma plaque only had significantly higher fasting blood sugar and hsCRP level and borderline higher PDAY risk score than those without plaque. To determine whether hsCRP and PDAY risk score might predict the presence of carotid atheroma plaque and compare the predictive power of these two markers, we used the area under the ROC curve with confidence interval. As Table 4 shows, both hsCRP level and PDAY risk score could predict the presence of carotid atheroma plaque. Furthermore, the PDAY risk score might independently predict the presence of carotid atheroma

Table 2. Characteristics of controls and individuals with familial hypercholesterolemia

Variable	Control (n = 22)	FH subjects (n = 23)
Age (years)	33 ± 12.9	27 ± 13.0
Male/female	11/11	17/6
Body mass index (kg/m^2)	22.5 ± 3.8	21.0 ± 4.8
Waist circumference (cm)	77.7 ± 8.0	77.0 ± 12.5
Systolic blood pressure (mmHg)	109 ± 10	112 ± 13
Diastolic blood pressure (mmHg)	65 ± 9	65 ± 11
Fasting blood glucose (mg/dl)	97.5 ± 18	90.9 ± 7
Total cholesterol (mg/dl)	191 ± 39.1	296 ± 80.4†
Triglyceride (mg/dl)	122 ± 65	113 ± 59
Low-density lipoprotein-cholesterol (mg/dl)	119 ± 33.1	218 ± 70.5†
High-density lipoprotein-cholesterol (mg/dl)	49 ± 11	56 ± 11
Total cholesterol/High-density lipoprotein cholesterol	3.9 ± 0.7	5.4 ± 1.5†
Apolipoprotein B (mg/dl)	95.3 ± 26.5	145.8 ± 43.4†
Apolipoprotein A1 (mg/dl)	130.6 ± 20.9	129.1 ± 27.4
PDAY risk score	12.1 ± 7.4	15.4 ± 9.4
High-sensitive C-reactive protein (mg/dl)	0.11 ± 0.09	0.10 ± 0.13
Carotid intima media thickness (cm)	0.10 ± 0.02	0.13 ± 0.04*

Values are means ± SDs or numbers of patients (percentages). *p < 0.05 vs. control subjects; †p < 0.001 vs. control subjects.

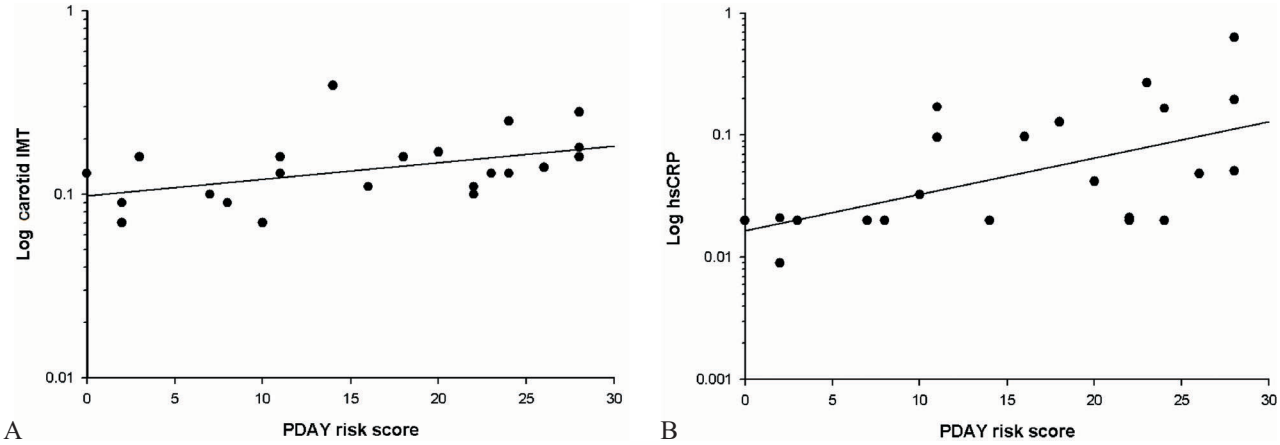

Figure 1. *(A) Semi-log scatter plot of the log carotid intima media thickness (y-axis) and PDAY risk score (x-axis). The solid line shows the correlation between carotid IMT and PDAY risk score. (r = 0.558, p < 0.001 by Spearman's correlation). (B) Semi-log scatter plot of the log high-sensitive C-reactive protein (y-axis) and PDAY risk score (x-axis). The solid line shows the correlation between hsCRP and PDAY risk score. (r = 0.430, p = 0.003 by Spearman's correlation). IMT, intima media thickness; hsCRP, high-sensitive C-reactive protein; PDAY, Pathobiological Determinants of Atherosclerosis in Youth.*

plaque by multivariate logistic regression (odds ratio for a 1-unit increase in the risk score was 1.161, 95% confidence interval: 1.027-1.312, p value: 0.017).

DISCUSSION

The current study demonstrated the aggravated atherosclerosis in these asymptomatic young heterozygous FH subjects, although these patients were relatively young and free of other major cardiovascular risk factors.[9] In addition, we demonstrated the relation between carotid IMT, inflammation markers and the PDAY risk score, a cardiovascular risk stratification tool for youth. Finally, we applied the PDAY risk score to predict the presence of carotid atheroma in FH subjects.

Table 3. Basic characteristics in FH subjects grouped by the presence of carotid atheroma plaque or not

Variable	Absence of carotid plaque (n = 11)	Presence of carotid plaque (n = 12)	p value
Age (years)	21.2 ± 12.3	33.1 ± 11.3	0.703
Male/female	7/4	10/2	0.288
Systolic blood pressure (mmHg)	109 ± 13	116 ± 13	0.849
Diastolic blood pressure (mmHg)	61 ± 8.4	69 ± 11.5	0.487
Body mass index (kg/m^2)	19.0 ± 3.5	22.9 ± 5.3	0.558
Waist circumference (cm)	70.3 ± 9.3	83.0 ± 12.3	0.930
Fasting blood glucose (mg/dl)	88.7 ± 2.4	92.8 ± 8.9	0.004
Total cholesterol (mg/dl)	293.6 ± 61.8	316.6 ± 76.5	0.149
Triglyceride (mg/dl)	132.0 ± 39.3	137.2 ± 65.0	0.225
Low-density lipoprotein-cholesterol (mg/dl)	183.4 ± 56.4	250.9 ± 68.4	0.263
High-density lipoprotein-cholesterol (mg/dl)	54.6 ± 12.9	57.9 ± 9.4	0.141
Apo lipoprotein B (mg/dl)	155.3 ± 35.8	164.5 ± 42.4	0.384
Apo lipoprotein A1 (mg/dl)	130.4 ± 22.0	137.1 ± 30.2	0.478
PDAY risk score	10.1 ± 8.3	20.3 ± 7.6	0.075
High-sensitive C-reactive protein (mg/dl)	0.04 ± 0.03	0.14 ± 0.17	0.021

Values are means ± SDs; FH, Familial Hypercholesterolemia; PDAY, Pathobiological Determinants of Atherosclerosis in Youth.

Table 4. The area under the ROC curve of PDAY risk score and high-sensitive C-reactive protein about predicting the presence of carotid atheroma plaque in FH subjects

Variable	Area under curve	p value	95% confidence interval
PDAY risk score	0.826	0.008	0.650-1.002
High sensitive C-reactive protein	0.761	0.034	0.564-0.959

FH, Familial Hypercholesterolemia; PDAY, Pathobiological Determinants of Atherosclerosis in Youth.

It has been demonstrated that high cholesterol burden and other cardiovascular risk factors exposure since childhood correlate to aggravated subclinical atherosclerosis in young adults.[10-13] Some studies have investigated the relation between the inflammation markers and atherosclerosis surrogate markers, including carotid IMT, coronary artery calcium and arterial stiffness in FH subjects.[2-4,14] To evaluate the cardiovascular disease risk in FH subjects, Hokins et al.[15] have evaluated a variety of cardiovascular risk factors. However, we need a simple and comprehensive method to make early risk stratification for the FH population.

The PDAY coronary artery risk score was developed to predict coronary artery lesions based on CHD risk factors determined at autopsy.[5] Previous studies showed that PDAY risk score also predicted carotid artery IMT and coronary artery calcium in living young to middle-age adults.[16,17] Chung et al. used this scoring system to predict subclinical atherosclerosis in women with systemic lupus erythematosus.[18] According to the study results, we demonstrated not only the predictive potential of PDAY score and carotid atheroma plaque, but also the correlation between inflammation marker and PDAY risk score in FH subjects. With this simple and non-invasive method, we can make a further risk stratification in FH subjects and it has been shown that early aggressive strategies using cholesterol-lowering therapy could modify the atherosclerosis process of those patients with high cardiovascular risk.[19,20]

Previous studies have investigated the relationship between serum LDL level and carotid IMT.[21] In this study, the baseline PDAY risk score did not differ significantly between FH and control subjects, and we did not note the correlation between serum LDL level and the presence of plaque in FH subjects. A limited study population might have contributed to the result. Furthermore, the atherosclerosis process might be determined by multiple risk factors, not only the LDL level.[22] The hypothesis of this study is to validate the multi-fractional evaluation system, the PDAY score, for cardiovascular

risk stratification in FH subjects. Despite a relatively small sample size, we still identified the predictive role of PDAY risk score for the atherosclerotic plaque in heterozygous young FH subjects.

CONCLUSION

In young FH subjects, those with carotid atheroma plaque had significant higher hsCRP and PDAY risk score. In multi-variate analysis, PDAY risk score was an independent predictor for the presence of carotid atheroma plaque in young FH subjects.

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