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Review Article

Pentraxin 3: A Novel Biomarker for Inflammatory Cardiovascular Disease

Kenji Inoue,[1] Tatsuhiko Kodama,[2] and Hiroyuki Daida[3]

[1] *Department of Cardiology, Juntendo University Nerima Hospital, Tokyo 177-0033, Japan*
[2] *RCAST, LSBM, The University of Tokyo, Tokyo 153-0041, Japan*
[3] *Department of Cardiology, Juntendo University School of Medicine, Tokyo 113-0033, Japan*

Correspondence should be addressed to Kenji Inoue, inouelsbm@gmail.com

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Numerous studies have recently examined the role of pentraxin 3 (PTX3) in clinical situations. The pentraxin family includes C-reactive protein (CRP); however, unlike CRP, PTX3 is expressed predominantly in atherosclerotic lesions that involve macrophages, neutrophils, dendritic cells, or smooth muscle cells. Interestingly, PTX3 gene expression in human endothelial cells is suppressed to a greater extent by pitavastatin than the expression of 6,000 other human genes that have been examined, suggesting that PTX3 may be a novel biomarker for inflammatory cardiovascular disease. The expression and involvement of PTX3 in cardiovascular diseases are discussed in this paper, along with the characteristics of PTX3 that make it a suitable biomarker; namely, that the physiological concentration is known and it is independent of other risk factors. The results discussed in this paper suggest that further investigations into the potential novel use of PTX3 as a biomarker for inflammatory cardiovascular disease should be undertaken.

1. Introduction

Biomarkers are measurable and quantifiable biological parameters that can have an important impact on clinical situations. Ideal biomarkers are those that are associated with disease clinical endpoints in observational studies and clinical trials, and in some cases, they may even be used as surrogate endpoints. Biomarkers must also be both independent of established risk factors and recognized to be a factor in the disease for which they are a marker. The normal physiological expression of a potential biomarker must also be known in order to interpret results, as well as to generalize results to various population groups. Finally, potential biomarkers must also have the ability to improve overall prediction beyond that of traditional risk factors, while assays to detect them must have an acceptable cost and be subject to standardization in order to control for the variability of measurements [1].

Basic research over the past decades has identified numerous candidate genes and proteins as biomarkers for cardiovascular disease. In the cardiovascular field, such biomarkers are useful not only for diagnosis but also as indicators of disease trait (risk factor or risk marker), disease state (preclinical or clinical), or disease rate (progression or prognosis) [2]. One protein that has the potential to be a viable biomarker for inflammatory vascular disease is pentraxin 3 (PTX3).

2. Pentraxin 3

PTX3 is an evolutionarily conserved, multimeric acute phase inflammatory glycoprotein in the same family as the well-established cardiovascular biomarker C-reactive protein (CRP) [3, 4]. PTX3 also shares 98% identity with tumor necrosis factor- (TNF-) stimulated gene 14 (TSG14) [5, 6]. PTX3 has been successfully identified by Breviario et al. using differential screening of a cDNA library from human umbilical vein endothelial cells (HUVECs) stimulated by interleukin-1 beta [5], as well as by Gustin et al. using the 2D-DIGE approach to detect PTX3 in HUVECs stimulated by lysophospholipids [7]. Our group also identified PTX3 when we were investigating statin as a target gene in HUVECs

incubated with pitavastatin for 24 hours prior to RNA extraction [8]. Interestingly, chip analysis has demonstrated that, of the 6,000 human genes that have been investigated for response to pitavastatin treatment, PTX3 gene expression is suppressed in human endothelial cells to the greatest extent.

PTX3 synthesis is stimulated in endothelial cells, macrophages, myeloid cells, and dendritic cells by cytokines and endotoxins such as bacterial products, interleukin-1, and TNF [9–11]. The role of PTX3 in neutrophils has also been gradually elucidated by a number of studies. Once synthesized, PTX3 is predominantly organized into covalent octamers through disulfide bonds [12]. Although PTX3 is mainly localized in lactoferrin positive-specific granules [13, 14], it is translocated to the surface of late apoptotic neutrophils upon stimulation, where it accumulates in blebs and is rapidly released. PTX3 then binds with the high-affinity complement component C1q to initiate the classical pathway of complement activation and facilitate pathogen recognition by macrophages.

3. Suitability of PTX3 as a Biomarker

3.1. PTX3 Expression in Cardiovascular Diseases

3.1.1. Acute Coronary Syndrome (ACS). The expression of PTX3 has been found to be increased in patients with acute myocardial infarction (AMI). For instance, Peri et al. observed that patients (n = 37) with AMI who were admitted to the coronary care unit within 3.2 ± 3.2 hours of the onset of symptoms had increased plasma PTX3 over time [15]. In this study, plasma PTX3 levels were found to peak at a median of 7.5 hours after AMI, and to return to normal levels after 3 days. Similarly, in murine models of AMI, PTX3 mRNA is expressed within 4 hours of the ligation of the coronary artery, reaches peak levels after 24 hours, and returns to normal levels 3 days later [16]. We have also found that plasma PTX3 levels are increased in patients (n = 16) with unstable angina pectoris (UAP; 6.20 ng/mL) [17]. Such findings have led to the investigation of PTX3 expression levels as a potential prognostic indicator of disease. Matsui et al. found that the expression of more than 3.1 ng/mL of PTX3 in patients with UAP/non-ST-elevation MI (n = 204) was predictive of the occurrence of a 6-month cardiac event, including cardiac death, rehospitalization for ACS, and rehospitalization for worsening heart failure [18], while Latini et al. have shown that the expression of more than 10.73 ng/mL of PTX3 predicted 3-month mortality in patients with AMI (n = 724) [19].

3.1.2. Congestive Heart Failure. PTX3 has also been implicated as a predictor of adverse clinical outcomes in patients with heart failure (n = 196) in a study with a median follow-up period of 655 days and an ejection fraction of less than 50% [20]. In a further study by Matsubara et al. that focused on patients with heart failure with normal ejection fraction (HFNEF), plasma PTX3 levels were also found to be increased (3.26 (2.36–4.35) ng/mL). This was observed even in patients with HFNEF, although B-type natriuretic peptide (BNP) was within normal limits [21].

3.1.3. Sleep Apnea Syndrome. Plasma PTX3 levels have also been suggested to be a good marker for the response to treatment of patients with obstructive sloop apnea (OSA). Kasai et al. demonstrated that not only did patients with OSA (n = 50) express higher levels of plasma PTX3 than individuals in an age- and body mass index-matched control group, but also that continuous positive airway pressure (CPAP) therapy led to a significant reduction in plasma PTX3 levels. While high sensitive CRP has previously been suggested to be a highly sensitive candidate biomarker that can reflect the status of patients with OSA, the findings of this study led the authors to conclude that plasma PTX3 levels seem to be a more suitable biomarker to monitor treatment effects in patients with OSA [22].

3.1.4. Heart Valvular Disease. In a study by Naito et al. that investigated PTX3 expression patterns in patients with aortic valve stenosis (AS) or regurgitation (AR), it was found that the expression of plasma PTX3 was significantly increased in patients with AS. Furthermore, PTX3 was found to be expressed predominantly in macrophage cells in the aortic valves of these patients [23].

3.2. PTX3 Involvement in Cardiovascular Diseases. Several studies have examined why plasma PTX3 levels are increased in patients with cardiovascular disease, and those that have targeted the PTX3 gene in mice suggest that plasma PTX3 levels may increase in order to confer protection against cardiac tissue damage [16, 24]. For instance, in a model of AMI caused by coronary artery ligation, PTX3-knockout mice showed exacerbated heart damage with a greater no-reflow area and increased inflammatory response, including increased neutrophil infiltration, a decreased number of capillaries, and an increased number of apoptotic cardiomyocytes [16]. This phenotype was reversed by the expression of exogenous PTX3.

PTX3 expression has also been examined using double knockout mice in which PTX3 and apolipoprotein E have been targeted. When gene expression in the aortic arches of these mice was analyzed using gene chip, it was found that several transcription factors involved in intracellular proinflammatory signaling, such as nuclear factor-kappa B and the related proteins Irak1, Fos, Jun, GATA3, GATA4, Egr2, and Egr3, were upregulated after the mice had been fed an atherogenic diet for 16 weeks. The mRNA expression levels of intracellular adhesion molecule, vascular cell adhesion molecule-1, endothelial leukocyte adhesion molecule-1, and platelet/endothelial cell adhesion molecule were also found to be increased in the vascular wall of double knockout mice when compared to those of wild-type mice [24]. Furthermore, the lack of PTX3 in a proatherogenic background may be associated with an increased inflammatory status in the vascular wall, which in turn contributes to the atherogenic process. In contrast, the transgenic overexpression of PTX3 has been found to result in greater resistance to lipopolysaccharide toxicity and cecal ligation and puncture [26]. There is also evidence that PTX3 may modulate inflammation-associated tissue damage.

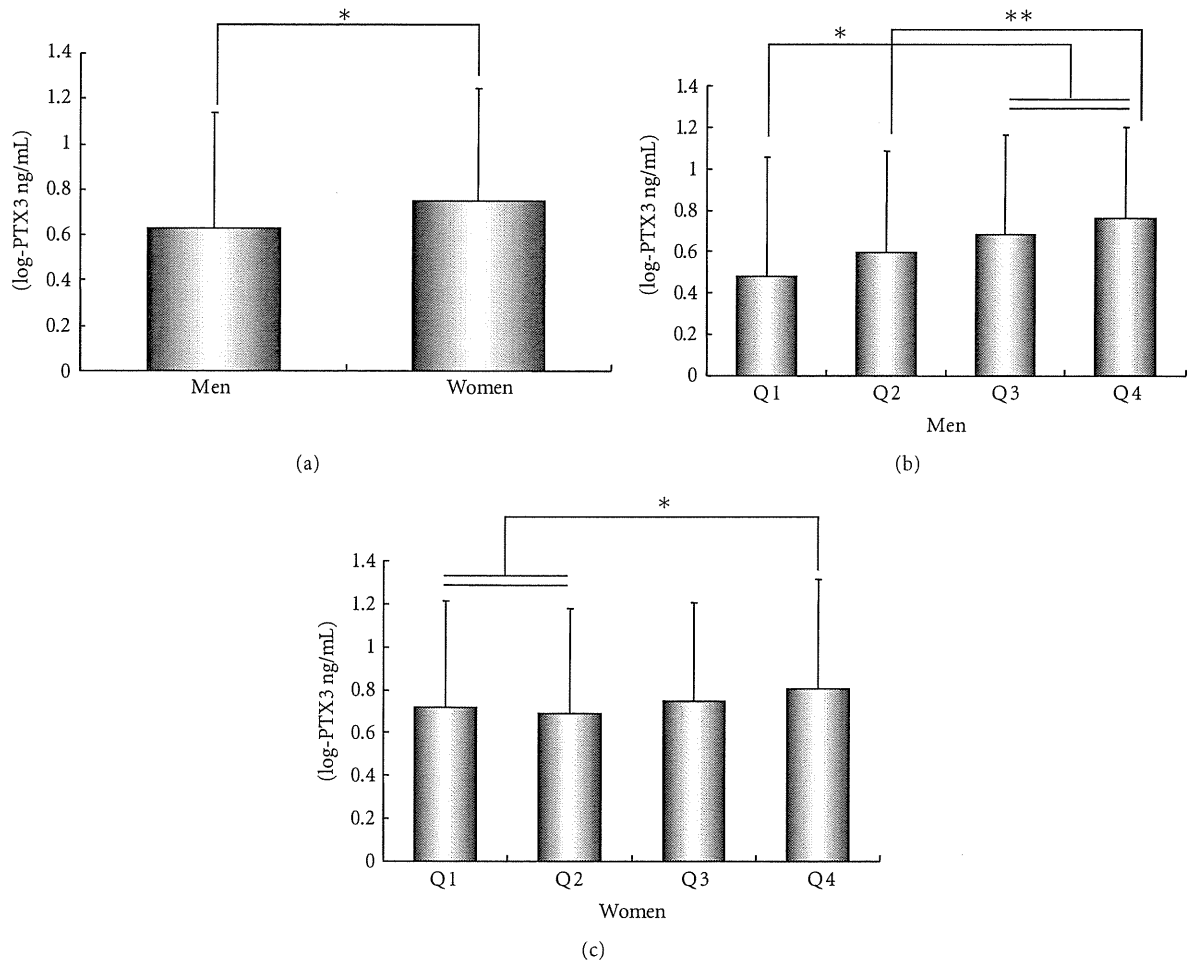

FIGURE 1: Geometric mean PTX3 plasma levels in men and women [25]. (a) Mean and confidence interval of natural log transformed PTX3 in men and women. Plasma PTX3 levels in men are significant lower than those in women (men, 1.87 (1.81, 1.94) ng/mL; women, 2.12 (2.05, 2.19) ng/mL). $*P < 0.0001$. (b) Plasma PTX3 levels according to quartiles of age in men. Quartile 1 (Q1): 37–49 years old; 1.62 (1.50, 1.74) ng/mL. Quartile 2 (Q2): 50–57 years old; 1.82 (1.70, 1.94) ng/mL. Quartile 3 (Q3): 58–68 years old; 1.98 (1.86, 2.11) ng/mL. Quartile 4 (Q4): 69–87 years old; 2.14 (2.02, 2.27) ng/mL. $*P < 0.001$, Q1 versus Q3 and Q4; $**P < 0.0006$, Q1 and Q2 versus Q4. (c) Plasma PTX3 levels according to quartiles of age in women. Quartile 1 (Q1): 38–52 years old; 2.05 (1.92, 2.18) ng/mL. Quartile 2 (Q2): 53–61 years old; 1.99 (1.87, 2.12) ng/mL. Quartile 3 (Q3): 62–70 years old; 2.10 (1.98, 2.23) ng/mL. Quartile 4 (Q4): 71–85 years old; 2.23 (2.02, 2.46) ng/mL. $*P < 0.05$, $**P < 0.01$.

PTX3 has also been found to offer protection against atherosclerosis. As a relationship between PTX3 and the cell adhesion molecule P-selectin in atherosclerotic lesions has recently been reported, it is possible that PTX3 may exert some of these effects through an association with this protein [27]. For instance, neutrophils rolling on P-selectin in venules at the sites of infection or injury receive signals that cause the release of PTX3 from specific granules. This released PTX3 then selectively binds locally expressed P-selectin, but not E- or L-selectin, in a paracrine manner, while the dissociation of this complex is slowed by the increased binding avidity due to the multimeric nature of PTX3. As more neutrophils roll, they release more PTX3, which then binds more P-selectin molecules. This constitutes a local negative feedback system that diminishes neutrophil tethering, accelerates rolling, and enhances detachment. Indeed, PTX3 expression has been found to decrease the number of neutrophils rolling on P-selectin *in vitro* in a concentration-dependent manner, while the injection of PTX3 *in vivo* has been shown to reduce the number of neutrophils rolling in thrombin-stimulated mesenteric venules of mice because PTX3 competitively inhibited between P-selectin and P-selectin glycoprotein 1 (PSGL-1) bonds.

The source of anti-inflammatory PTX3 has also been examined. By transplanting wild-type or PTX3-deficient bone marrow into irradiated wild-type or PTX3-deficient recipient mice, Deban et al. showed that PTX3 from hematopoietic cells is required to suppress neutrophil recruitment into the pleural cavity in the first 2 hours after chemokine challenge. In this short time frame, neutrophils are the likely source of PTX3, as they are the only hematopoietic cells that store PTX3 [28].

Very recently, Maugeri et al. have reported data that supports the release of PTX3 from activated neutrophils by platelets in patients with ACS [28]. In this study, the total amount of PTX3 in the neutrophils of patients with early

AMI (early onset; <6 hr), late AMI (<48 hr), stable coronary artery disease, and healthy volunteers was measured using FACS. As found in our study, the maximum plasma level of PTX3 was reached at 6 hours after onset. Interestingly, the lowest PTX3 levels were found in the neutrophils of patients with early AMI, whereby confocal microscopy detected very low PTX3 expression in neutrophils from patients with early AMI and much higher PTX3 expression in neutrophils from patients with late AMI. Furthermore, released PTX3 from patients with early AMI was found to aggregate platelets expressing P-selectin compare with late AMI [28]. From these findings, PTX3 works as a cardioprotective to bind to activated circulating platelets and reduce the inflammation status in cardiovascular bed.

It has also been shown that plasma PTX3 levels increase significantly during widespread inflammations, such as sepsis [29]. In such scenarios, activated endothelial cells, dendritic cells, and/or macrophages may be major sources of PTX3, and although it has recently been demonstrated that PTX3 inhibits P-selectin-dependent adhesion [27], other, still undefined, mechanisms may also contribute to its anti-inflammatory properties *in vivo*.

3.3. Physiological PTX3 Levels. The normal physiological concentration of plasma PTX3 expression has been determined to be approximately 2 ng/mL in a study that examined PTX3 levels in 1749 subjects (818 men and 931 women) [25]. Interestingly, plasma PTX3 levels were found to be significantly lower in men than in women (1.87 (1.81, 1.94) ng/mL versus 2.12 (2.05, 2.19) ng/mL, $P < 0.0001$) (Figure 1(a)). They were also found to be significantly higher in the oldest age group in both men and women (lowest quartile 1.62 (1.50, 1.74) ng/mL versus highest quartile 2.14 (2.02, 2.27) ng/mL in men, $P < 0.001$; lowest quartile 2.05 (1.92, 2.18) ng/mL versus highest quartile 2.23 (2.02, 2.46) ng/mL in women, $P < 0.05$; Figures 1(b) and 1(c)). PTX3 levels were also inversely correlated with triglyceride levels ($r = -0.19$ in men and $r = -0.18$ in women, $P < 0.00001$), and body mass index ($r = -0.16$ in men and $r = -0.24$ in women, $P < 0.00001$).

3.4. PTX3 Independence from Established Risk Factors. Plasma PTX3 levels have also been shown to be independent of other coronary risk factors, including total cholesterol, high-density lipoprotein (HDL) cholesterol, hemoglobin A1C, smoking status, gender, and obesity (Table 1) [17]. Although Yamashina et al. have reported a brachial-ankle pulse wave velocity (ba PWV) cutoff value of 14.0 m/s for screening subjects at risk of developing cardiovascular diseases in the general population [30], plasma PTX3 levels are not different between patients with ba PWV values of more or less than 14.0 m/s, or an intimal thickness of the carotid artery of more or less than 1.0 mm, which means within normal limits [17].

Table 1: Geometric mean PTX3 plasma levels by CHD risk factors [17].

Risk factor		PTX3 (ng/mL; 95% CI)	*P* value
TCHO	≧220 mg/dL	2.16 (1.85–2.46)	0.51
	<220 mg/dL	2.30 (2.01–2.60)	
HDL	≧40 mg/dL	2.23 (1.98–2.48)	0.42
	<40 mg/dL	2.03 (1.62–2.43)	
HgbA1C	≧5.9%	2.12 (1.83–2.42)	0.29
	<5.9%	2.36 (2.02–2.66)	
Obesity	≧24.2 kg/m^2	1.98 (1.64–2.32)	0.07
	<24.2 kg/m^2	2.39 (2.12–2.67)	
IMT	≧1.0 mm	2.30 (2.02–2.58)	0.56
	<1.0 mm	2.24 (1.80–2.68)	
Smoke	Smoking	2.32 (1.96–2.68)	0.58
	None	2.20 (1.93–2.47)	
Gender	Male	2.26 (1.97–2.54)	0.84
	Female	2.22 (1.93–2.51)	

CHD: coronary heart disease.
TCHO: total cholesterol; HDL: high-density lipoprotein; HgbA1C: hemoglobin A1C; IMT: intimal media thickness.
CI: confidence interval.

4. PTX3 in Other Diseases

The human PTX3 proximal promoters contain AP-1, NF-kappa B, Sp-1, and NF-IL6 binding sites [5]. Consequently, PTX3 is expressed in response to proinflammatory signals, including bacteria, IL-1 (but not IL-6), and TNF-alpha produced by primarily endothelial cells, neutrophils, and macrophages. As a result, inflammation diseases, especially disorders of the immune system such as rheumatoid arthritis [31], progressive systemic sclerosis [32], Chug-Straus syndrome, Wegener's granulomatosis, and microscopic polyangiitis [33], as well as systemic inflammatory response syndrome (SIRS) [29, 34], result in increased expression of plasma PTX3. Chronic kidney disease is also known to increase the level of plasma PTX3 [35, 36]. Therefore, it was also of interest to determine the PTX3 expression patterns in inflammatory bowel diseases such as Crohn's disease and ulcerative colitis. As IL-6 was found to have increased expression in active Crohn's disease, but not in ulcerative colitis, it is not surprising that plasma PTX3 levels were increased in patients with only ulcerative colitis (because IL-1, but not IL-6, causes induction of PTX3 expression). PTX3 may therefore also be a good diagnostic marker for deterioration in patients with inflammatory bowel disease [37, 38].

5. Conclusion

Advances in genomics and proteomics technologies have led to the discovery of many novel biomarkers that provide valuable information, which can be used in disease screening and diagnosis, determining prognoses, and therapeutic monitoring. One potentially useful biomarker for cardiovascular disease is PTX3, and many studies have recently examined

this protein in clinical situations. Although PTX3 is in the same protein family as CRP, it is expressed predominantly in atherosclerotic lesions. Interestingly, the expression of PTX3 in endothelial cells has been shown *in vitro* to be suppressed to a greater extent by pitavastatin than other genes. We have therefore recently determined the normal physiological concentration of PTX3. As PTX3 has promise as a biomarker for cardiovascular disease, we have recently determined the normal physiological concentration of this protein. In addition, kits capable of detecting PTX3 are available, including a highly sensitive kit recently developed by our group, facilitating the use of PTX3 as a biomarker. Additional clinical study will be necessary to further elucidate the role of this protein in cardiovascular disease.

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Special Report

Background to Discuss Guidelines for Control of Plasma HDL-Cholesterol in Japan*

Shinji Yokoyama[1], Shizuya Yamashita[2], Shun Ishibashi[3], Hirohito Sone[4], Shinichi Oikawa[5], Kohji Shirai[6], Takao Ohta[7], Hideaki Bujo[8], Junji Kobayashi[9], Hidenori Arai[10], Mariko Harada-Shiba[11], Masaaki Eto[12], Toshio Hayashi[13], Takanari Gotoda[14], Hiroaki Suzuki[15] and Nobuhiro Yamada[16]

[1]Food and Nutritional Sciences, College of Bioscience and Biotechnology, Chubu University, Kasugai, Japan
[2]Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Suita, Japan
[3]Division of Endocrinology and Metabolism, Diabetes Center, Department of Medicine, Jichi Medical University Graduate School of Medicine, Shimotsuke, Japan
[4]Department of Endocrinology and Metabolism, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan
[5]Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan
[6]Internal Medicine, Sakura Hospital, School of Medicine, Toho University, Sakura, Japan
[7]Department of Child Health and Welfare (Pediatrics), Faculty of Medicine, University of the Ryukyus, Nishihara, Japan
[8]Department of Genome Research and Clinical Application, Chiba University Graduate School of Medicine, Chiba, Japan
[9]Department of Lipidology, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan
[10]Department of Human Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan
[11]Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan
[12]School of Pharmaceutical Sciences, Ohu University and Department of Medicine, Ohu University Hospital, Kohriyama, Japan
[13]Department of Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan
[14]Department of Clinical and Molecular Epidemiology, 22nd Century Medical and Research Center, University of Tokyo Hospital, Tokyo, Japan
[15]Department of Endocrinology and Metabolism, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan
[16]University of Tsukuba, Tsukuba, Japan

A decrease in high density lipoprotein-cholesterol (HDL-C) is a strong risk factor for atherosclerotic disorders in Japan, probably more important than an increase in low density lipoprotein-cholesterol (LDL-C). While there are rational grounds for the argument that elevation of HDL-C leads to decreased risk, there has as yet been no direct evidence of such an effect. If elevation of HDL-C decreases the risk, this effect is expected throughout the normal range of HDL-C or perhaps even higher than that. Simulation based on epidemiological data indicated that it may eventually reduce the incidence of ischemic heart disease by 60-70% in Japan. In the risk management guideline, "low" HDL-C is presently defined as 40 mg/dL or below. While there is no evidence that strongly urges a change in this definition, the results of epidemiological studies support "The higher the HDL-C level, the lower the risk," even in the "normal range". Elevation of the HDL-C level may reduce the risk, probably at least up to 70 mg/dL; however, there are no supportive data for this effect still being obtained over 80 mg/dL. Patients with homozygous CETP deficiency should be followed-up while controlling other risk factors, so as not to dismiss the possibility of a risk increase with an extremely elevated HDL-C level.

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Key words; HDL, LDL, Guidelines, NNT, Prevention

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Address for correspondence: Shinji Yokoyama, Food and Nutritional Sciences, College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai, Japan
E-mail: syokoyam@med.nagoya-cu.ac.jp
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Clinical Relevance of HDL-C Management

Numbers of epidemiological studies have established that the risk of coronary artery disease increases as plasma HDL-C decreases, and decreases as it increases. In addition, many experimental approaches

have demonstrated that cholesterol is extracted by HDL particles in the culture medium from cultured cells, including macrophages overloaded with cholesterol.

From these two lines of evidence, HDL is believed to be a "preventive factor" against atherosclerosis. This view is strongly associated with the hypothesis that HDL plays a central role in the recovery of cholesterol molecules from tissues and organs, which cannot be catabolized in peripheral cells, and in their transport to the liver for conversion to bile acids. From the viewpoint of public health, many research results suggest that a decrease in HDL-C contributes more than an increase in LDL-C to the development of ischemic heart disease in Japan. In studies conducted at Nagoya City University, for example, narrowing of the coronary artery was more closely related to triglycerides (TG) and HDL-C than to total cholesterol (TC) or LDL-C[1, 2], and this tendency is commonly observed in many other reports. HDL-C is thus suggested to be a strong determinant of atherosclerosis in Japan and perhaps a more important risk factor than LDL-C from a public health point of view.

HDL is smaller (12 nm or less in diameter) than other lipoproteins, abundant in protein and does not contain much TG, so it has a greater hydrated density than other lipoproteins (d=1.063-1.21). Similarly to other plasma lipoproteins, however, HDL functions to transport cholesterol among cells or organs using the flow of blood or extracellular fluid. Cholesterol, an essential molecule for the life of animals, requires a number of steps and plenty of energy for synthesis, and its dietary intake is not always guaranteed; therefore, the animal body has developed systems to use cholesterol sparingly as a precious material. As a result, little cholesterol is converted to energy in its catabolism, and, with the exception of a very small amount used for the production of steroid hormones, most cholesterol is transported to the liver for conversion to bile acids and is recycled an reused in the intestine before excretion. Its steroid backbone is not degraded in the metabolism in the animal body and finally broken down by microorganisms in the environment. Therefore, cholesterol molecules must be released from most somatic cells for metabolic homeostasis, and HDL receives these cholesterol molecules for their transport. Cholesterol is converted to cholesteryl acylester (CE) as a fatty acyl chain and transferred from phosphatidylcholine to its hydroxyl group to form an ester bond, for packing cholesterol molecules into the core of HDL. CE is recovered by the liver directly from HDL by a selective uptake reaction, or as LDL particles after being transferred to apolipoprotein B-containing lipoproteins by CE transfer protein (CETP). As a result of these activities, HDL is considered to exert a preventive effect against atherosclerosis as it interferes with the excessive accumulation of cholesterol in cells from LDL, etc., by extracting it.

No drug has been marketed yet to independently increases HDL-C; therefore, the question of whether increasing HDL-C is effective for preventing and treating atherosclerotic disorders has not been answered. However, researchers have recently directed more attention to HDL and, accordingly, more research results on HDL metabolism have recently accumulated. Much effort to develop drugs targeting HDL has been initiated. On the other hand, some existing drugs are known to increase plasma HDL-C. Drugs that reduce TG generally increase HDL-C, primarily because these drugs reverse low HDL-C induced by high TG through CETP[3]. In addition, fibrates have been suggested to directly increase HDL production[4]. Many clinical studies have also shown that statins elevate HDL-C as well as decreasing LDL-C. Concerning their mechanism, statins have recently been reported to increase HDL synthesis in the liver, unlike their effects in peripheral tissues[5]. The mechanism of the increase in HDL through exercise and alcohol intake has not been sufficiently elucidated. As mentioned below, the question of whether HDL-C increase by inhibiting CETP prevents atherogenesis has been shelved because of the failure to develop a CETP-inhibiting drug, perhaps due to a business-oriented strategy[6].

Position of HDL in Risk-Reducing Strategies

Large-scale clinical studies targeted to high LDL-C and high TG, major risk factors of atherosclerotic diseases, such as ischemic heart disease, have indicated that ischemic heart disease can be prevented by reducing LDL-C and TG and, particularly, that mortality due to the disease can be lowered by controlling the LDL-C level, with a consequent reduction in the total number of deaths in the high-risk group. In addition, based on stratified analysis of the results of many clinical trials, the conclusion has been reached that an increase in HDL-C contributes to the prevention of diseases as a "statistically independent factor". In consideration of the above-stated marked epidemiological contribution of HDL-C as a "negative risk factor" and the significant "indirect evidence" of an increase in HDL-C in the prevention of atherogenesis, the argument that a standard should be set for the control of HDL-C appears to be well grounded. However, it is also true that a consensus concerning

HDL-C management, similar to that in evidence-based quantitative guidelines for the control of LDL-C and the management and treatment of high TG, is difficult to reach at present, when no therapeutic technique specifically targeted to increase HDL-C has reached a practical level and there is no direct evidence concerning the prevention and treatment of atherosclerotic disorders using such a technique. Thus, any therapeutic guideline regarding HDL-C is merely a "proposal" based on indirect circumstantial evidence until the results of a large-scale clinical trial of a technique to specifically increase HDL-C become available.

Recently, some negative implications have been spread regarding the anti-atherosclerotic effect of an increase in HDL-C, inviting some confusion in the discussion. One is the discontinuation of a large-scale clinical study on the prevention of ischemic heart disease by increasing HDL-C, carried out to develop the CETP inhibitor torcetrapib, due to an increase in the mortality rate in the treated group[6]. Another is a large-scale epidemiological study reporting that a mutation to cause dysfunction of ABCA1, a rate-regulating protein of HDL biogenesis, is not likely to be a risk factor of ischemic heart disease[7]. The first report appears to support the contention of researchers arguing that "an increase in HDL-C by CETP inhibition has no anti-atherosclerotic effect," and allowed the generalized assertion that "the HDL-C increasing strategy is a mistake" to emerge; however, these reports do not necessarily mean the failure of CETP inhibitors themselves, and the pressor effect of a particular drug, torcetrapib, is likely to have led to such results. This incidence postponed an answer to the question of whether increasing HDL-C with a CETP inhibitor is a good idea, the most important medical issue, and markedly complicated the strategy for developing HDL-C elevating agents in general. Also, studies on ABCA1 mutation have shown that the maximum decrease in HDL is about 20%, suggesting that this does not necessarily reject the benefit of high HDL-C.

Under these circumstances, the position has not changed that an elevation of HDL-C is an important part of the anti-atherosclerotic strategy, including CETP inhibition. The above discussion may be summarized as follows: 1) a decrease in HDL-C is a strong risk factor for atherosclerotic disorders, 2) there are rational grounds for the supposition that this risk can be reduced by correcting low HDL-C (increasing HDL-C), but 3) no direct evidence has been obtained that increasing HDL-C is effective for the prevention and treatment of atherosclerotic disorders, 4) changes in HDL-C may include changes in the number and size of HDL particles, and the difference in their clinical significance may become a problem in the future.

Simulation of Atherosclerosis Prevention by Increasing HDL-C

There are qualitative scientific grounds for lowering the LDL-C level to reduce the risk of atherosclerotic disorders or, more specifically from an evidence-based viewpoint, to reduce the probability of the occurrence of ischemic heart disease; however, to prepare specific guidelines for diagnosis and treatment, quantitative criteria are considered indispensable. This is a problem with the concept in setting therapeutic goals for target groups. A quantitative profile of increases in the risk associated with elevations of the LDL-C level is necessary, and, if possible, results directly showing that the treatment reverses this curve of increasing risk must be presented. It is not impossible to set medical goals according to this parameter alone, but how criteria are set markedly affects the cost-effectiveness of treatment depending on the distribution of the HDL-C level and demographic composition of the target population; therefore, simulation involving these factors is one of the tasks that must be implemented to devise guidelines.

Fig. 1B shows the relationship between the LDL-C level and incidence (per 1,000 people) of myocardial infarction (lethal/non-lethal) in the JLIT, a cohort study that followed up a simvastatin-treated group for 5 years[8]. From this graph, the distribution of the HDL-C level in Japanese of corresponding ages (**Fig. 1A**)[9], and the population composition of the Japanese by age, the number of people needed to treat (NNT) and number of patients in whom the disease is prevented can be calculated when the control target is fulfilled 100% by reducing LDL-C (**Fig. 1C**). According to this calculation, the primary prevention efficacy, expressed as the inverse of NNT, is high at a target LDL-C level of 140 mg/dL but begins to fall rapidly as it is reduced to 120 mg/dL. Reflecting this, the incidence of myocardial infarction shows no further decrease when the target control level is set lower than 140 mg/dL. According to this analysis, roughly 140 mg/dL is considered to be medically and medico-economically appropriate as the target control level of LDL-C for primary prevention, at least on the basis of the results of the JLIT. In this case, the maximum preventive effect is 30-35% for myocardial infarction, which is in close agreement with the results of the MEGA study, the only large-scale interventional study of ischemic heart disease conducted in Japan using a statin[10].

Fig. 2B shows the decreases in the risk of ischemic heart disease associated with elevations of the HDL-C level in 3 epidemiological studies with prospective risk evaluation carried out in Japan including the JLIT[8, 11, 12]. While it is difficult to directly compare the incidences because the clinical definition of the endpoint varied among the studies, the peak decrease of the risk associated with increased HDL-C is less notable than that associated with the change of LDL-C in all studies. In other words, HDL-C-dependent decreases in the risk were observed even at HDL-C exceeding 60 mg/dL in all 3 studies. **Fig. 2C** shows the results of simulation similar to that of LDL-C performed using the results of the JLIT, which analyzed the therapeutic outcomes, on the basis of the HDL-C distribution curve in Japanese (**Fig. 2A**)[9] and the population composition. Since decreases in the risk associated with increases in HDL-C have not been directly demonstrated, the simulation was based on the hypothesis that increases in the risk associated with decreases in HDL can be reversed by increasing HDL-C. In contrast with the results concerning LDL, little decrease or peaking of the preventive efficacy associated with increased HDL-C was observed with an HDL-C level over 60 mg/dL. Reflecting this, the preventive effect against myocardial infarction could still be increased by raising the HDL-C level beyond 60 mg/dL. These results suggest that, under the hypothesis that the risk of myocardial infarction is reversibly reduced by elevating HDL-C, myocardial infarction can be prevented in 60-70% of the Japanese population at risk.

As far as these results are concerned, it can be concluded that the criterion of a "low HDL-C level" is unnecessary in guidelines for the control of HDL-C, and that the higher the HDL-C the better; however, according to the results in **Fig. 2A**, some studies have shown relatively large increases in the risk associated with decreases in HDL-C at about 50 mg/dL or below and, particularly, below 40 mg/dL; therefore, it may be reasonable to set a "caution level" around here. On the other hand, views on high HDL-C are divided. First, there is no epidemiological evidence indicating that higher HDL-C is better, even when it exceeds 60 mg/dL. This is probably because the population falling in this category is small (even though high HDL-C is relatively frequent in Japan) and cardiovascular incidence is low, making it difficult to obtain significant results.

In addition, the controversy is further complicated by the inclusion in this category of cases of homozygous CETP-deficient patients, in which elevations of HDL may not be considered to decrease the

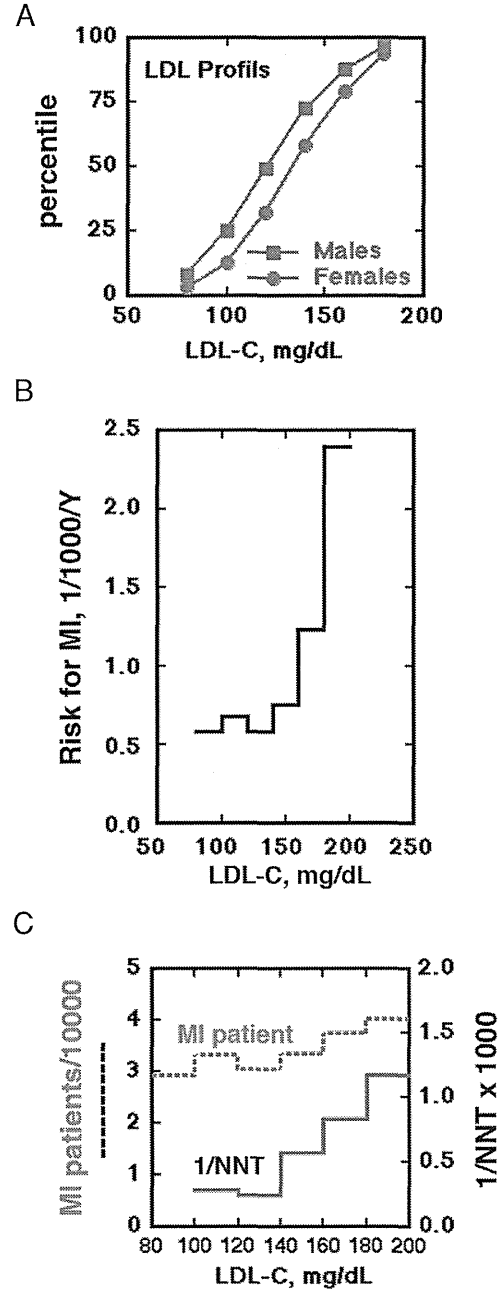

Fig. 1. Prevention of ischemic heart disease in Japanese by reducing LDL.

A: Distribution curve of the plasma LDL-C level in Japanese[9]. B: Relationship between the plasma LDL-C level and risk of "myocardial infarction" observed in the JLIT[8]. C: Simulation of the prevention of "myocardial infarction" based on Graphs A and B and demographic data for Japanese. Solid lines represent the inverse of NNT (·1,000) as an indicator of the treatment efficacy for managing lipoproteins to a target. The value of each horizontal segment is the efficacy when reaching a target LDL-C value at the left end of the segment in all Japanese at ages covered by the JLIT. Each horizontal segment of broken lines represents the number of MI patients when LDL is reduced to or lower than the level of the right end of the segment.

risk. The argument that increased HDL does not necessarily contribute to decreased risk is supported by the absence of a further decrease in the risk when the HDL-C increases above 70 mg/dL and the increased risk in patients with a homozygous CETP defect[13]; however, HDL-C is usually 80 mg/dL or higher and often reaches 100-200 mg/dL or even higher in patients with a homozygous CETP defect[13-16], and such high HDL-C should be considered separately from regular high HDL-C. Still, researchers are not in agreement concerning the increase in risk. In this sense, the differentiation of homozygous CETP deficiency is necessary in patients showing HDL-C exceeding 80 mg/dL, and there is no clinical or experimental evidence pointing to any conclusion about whether HDL-C should be maintained above this level. Nevertheless, the high prevalence of CETP deficiency among Japanese (1/20 for D442G and 1/100 for I14A) may have a limited but significant impact on the association between high HDL and atherogenesis in Japanese.

Proposal of Standards for Management of the HDL-C Level

On the basis of the above discussion, this article summarizes a proposal for the management of the HDL-C level as follows:

1) The evidence status is summarized as (1) A decrease in HDL-C is a strong risk factor for atherosclerotic disorders, particularly in Japan and, from the viewpoint of public health, it may be a more important risk factor than an increase in LDL-C; (2) While there are rational grounds for the argument that elevated HDL-C leads to a decreased risk, (3) there is as yet no direct evidence that elevating HDL-C is effective for the prevention and treatment of atherosclerotic disorders.

2) If elevations of HDL-C through interventional measures cause reversible decreases in the risk, this effect is expected, at least, up to 60 mg/dL or higher, and a simulation indicated that it eventually reduce the incidence of ischemic heart disease in Japan by 60-70%.

3) In risk management, high HDL-C is presently defined as 40 mg/dL or below. While there is no evidence that strongly urges a change in this definition, the results of epidemiological studies support "the higher the HDL-C level, the lower the risk," even in the "normal range" so that elevation of HDL-C may reduce the risk probably at least up to 70 mg/dL; however, there are no supportive data for this effect still being obtained over 80 mg/dL. Patients with a

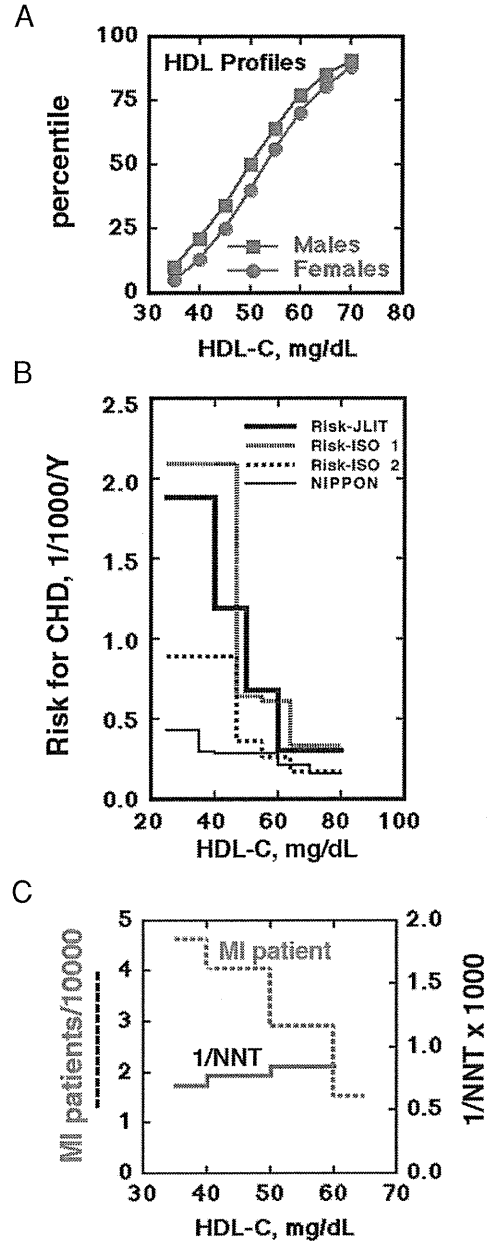

Fig. 2. Prevention of ischemic heart disease in Japanese by increasing HDL-C.

A: Distribution curve of the plasma HDL-C level in Japanese[9]. B: Relationship between the plasma HDL-CH level and risk of ischemic heart disease in Japanese. "Myocardial infarction" in the JLIT[8], "coronary artery disease" and "definitive diagnosis of myocardial infarction" by Kitamura, Iso, *et al.*[11], and "deaths due to cardiovascular diseases" according to NIPPON DATA[12]. C: Simulation for prevention of "myocardial infarction" based on Graphs A and B and demographic data of Japanese. Solid lines represent the inverse of NNT (x 1000) as an indicator of the treatment efficacy for managing lipoproteins to a target. The value of each horizontal segment is the efficacy when reaching a target HDL level at the right end of the horizontal segment in all Japanese at ages covered by the JLIT. Each horizontal segment of broken lines represents the number of MI patients when HDL is raised to the left end of the segment.

homozygous CETP deficiency should be followed-up while controlling other risk factors, not to dismiss the possibility of the risk increase with an extremely elevated HDL-C level. A gender-dependent strategy for HDL-C management should be discussed when further epidemiological and clinical evidence becomes available.

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Original Article

Defining Patients at Extremely High Risk for Coronary Artery Disease in Heterozygous Familial Hypercholesterolemia

Takako Sugisawa[1], Tomonori Okamura[2], Hisashi Makino[1], Makoto Watanabe[3], Ichiro Kishimoto[1], Yoshihiro Miyamoto[3], Noriyuki Iwamoto[1], Akira Yamamoto[4], Shinji Yokoyama[5] and Mariko Harada-Shiba[1, 6]

[1]Division of Endocrinology and Metabolism, National Cerebral and Cardiovascular Center, Japan
[2]Department of Public Health and Preventive Medicine, Keio University, Japan
[3]Department of Preventive Cardiology, National Cerebral and Cardiovascular Center, Japan
[4]Health Care Facilities for the Aged, Minoh Life Plaza, Japan
[5]Food and Nutritional Sciences, College of Bioscience and Biotechnology, Chubu University, Japan
[6]Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan

Aim: Heterozygous patients with familial hypercholesterolemia (FH) are known to be associated with a high risk of coronary artery disease (CAD), which is a major determinant of their clinical outcome. The prognosis of heterozygous FH patients substantially varies, being dependent on the level of their CAD risk, and their therapeutic regimen should be individualized. We assessed critical levels of LDL-cholesterol (LDL-C) and Achilles tendon thickness (ATT) to identify heterozygous FH patients at "very high" risk for CAD.
Methods: One hundred and nine heterozygous FH patients who had no history of CAD and had had their plasma lipid profile and ATT assessed before treatment were followed up until their first CAD event or 31 December 2010. Multivariable logistic regression models were used to analyze the correlation of LDL-C and/or ATT levels with the risk of developing CAD.
Results: During the follow-up period, 21 of the 109 patients had a CAD event, diagnosed by coronary angiogram. Individuals in the highest tertile of LDL-C had a CAD risk 8.29-fold higher than those in the lowest tertile. Individuals in the highest tertile of the ATT group had a 7.82-fold higher CAD risk than those in the lowest tertile. Those who had either LDL-C ≥ 260 mg/dL or ATT ≥ 14.5 had a 23.94-fold higher CAD risk than those with LDL-C < 260 mg/dL and ATT < 14.5 mm.
Conclusions: In heterozygous FH patients, LDL-C 260 mg/dL or higher and/or ATT 14.5 mm or thicker are useful markers for extracting patients at "very high" risk for CAD.

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Key words; Familial hypercholesterolemia, LDL cholesterol, Coronary artery disease, Coronary risk, Achilles tendon thickness

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by hypercholesterolemia, skin and tendon xanthomas and a high risk of coronary artery disease (CAD) due to premature atherosclerosis[1)]. FH has the highest prevalence in genetic metabolic diseases, showing one per 300 to 500 heterozygous patients in the general population[1, 2)]. High low-density lipoprotein cholesterol (LDL-C) is the first symptom, appearing in heterozygous FH even from birth, and xanthomas in the Achilles tendon usually appear during or after the late 10s and are found in half of all patients by the age of 30[1)]. Coronary artery disease (CAD), which determines the prognosis of FH patients, appears during or after the third decade of life in men and the fifth decade in women[3-5)].

Address for correspondence: Mariko Harada-Shiba, Department of Molecular Innovation in Lipidology, National Cerebral and Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka, 565-8565, Japan
E-mail: shiba.mariko.ri@mail.ncvc.go.jp
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CAD mortality in heterozygous FH is several times higher than that in the general population[1, 6-8]; therefore, it is very important to prevent CAD in heterozygous FH patients. The prognosis of heterozygous patients of FH varies substantially, such that some develop CAD at their 20s while others may not develop CAD until their seventh decade; therefore, the therapeutic regimen should be individualized according to the patients' risk of CAD.

Various risk factors for CAD have been identified in heterozygous patients with FH, such as age, sex, LDL-C, triglyceride (TG), HDL-C, Achilles tendon thickness (ATT), smoking, a family history of CAD, hypertension, diabetes mellitus, Lp(a), homocysteine and so on[3, 9-12]. Among these parameters, LDL-C and ATT are simple, specific and non-invasive to measure, and can easily be used by primary care physicians to evaluate the CAD risk. We therefore estimated the CAD risks in accordance with LDL-C and ATT in heterozygous FH patients in order to identify those at extremely high risk.

Methods

Subjects

Of the patients referred to the lipid clinic at the National Cerebral and Cardiovascular Center (NCVC) from 1977 to 2007, 329 consecutive patients diagnosed as FH heterozygotes using previously described criteria[6] were subjected to this study. After diagnosis, the FH patients had medical checks according to the standard procedure for treating heterozygous FH in NCVC. The patients were subjected to a treadmill test for CAD screening just after their first visit to our clinic. Those who had a positive result on the treadmill test were subjected to a coronary angiogram (CAG), and diagnosed with CAD with 75% or more stenosis. Those who had a negative result on the treadmill or no significant stenosis by CAG were included in this study. Among the 329 FH patients, 229 were excluded: 53 had a past history of CAD, 160 had not had LDL-C measured before treatment, 76 had not had ATT thickness measured and 3 had TG more than 4.5 mmol/L, so 109 were followed up until their first CAD event or 31 December 2010. After the first visit to our clinic, dietary and drug treatment, including statins, was immediately started and continued.

During the course, those who had chest pain or a positive result on the treadmill test performed biennially were subjected to CAG, and diagnosed with CAD with 75% or more stenosis. Medical records of the patients were examined according to the analysis protocol approved by our institutional ethics committee (ID#M20-25-2).

Clinical and Laboratory Characteristics

Serum lipid and lipoprotein levels were measured at the time of initial diagnosis, before any lipid-lowering treatment. TC, TG and HDL-C levels were measured enzymatically with a commercial kit (Daiichi Pure Chemicals Co., Tokyo, Japan) using an automated analyzer (Hitachi model 704; Hitachi, Tokyo, Japan) in the clinical laboratory of the National Cerebral and Cardiovascular Center (NCVC). LDL-C was calculated by the Friedewald formula. ATT was measured by X-ray according to the method previously described[13]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Hypertension was defined as the use of antihypertensive drugs or blood pressure ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic or both at the first clinic visit (the criteria for hypertension of the Japanese Society of Hypertension Guidelines)[14]. Diabetes mellitus was defined according to the 2002 Guideline for the Treatment of Diabetes Mellitus of the Japanese Diabetes Society[15]. A family history of CAD was defined as positive by having within 2nd degree family members with CAD on the standardized questionnaire. Smoking was defined as positive by having a smoking habit at the first visit to NCVC on the patient report.

Statistical Analyses

Continuous variables are presented as the means ± SDs. Categorical data are presented as numbers and percentages. Unpaired Student's *t*-test and one-way analysis of variance (ANOVA) were used to assess differences between groups in continuous variables. Differences in categorical variables were assessed by the χ^2 test.

Multivariable logistic regression analysis after adjusting for age, sex, hypertension, diabetes mellitus, smoking, family history of CAD, and low HDL-C (<40 mg/dL) were used to analyze correlations of LDL-C levels or ATT levels and the development of CAD. LDL-C levels were categorized into tertiles: (1) LDL-C <206 mg/dL, (2) LDL ≥206 and <260 mg/dL, (3) LDL-C ≥260 mg/dL. ATT levels were also categorized into tertiles: (1) ATT <9.0 mm, (2) ATT ≥9.0 mm and <14.5 mm, (3) ATT ≥14.5 mm. All the confidence intervals were estimated at the 95% level and significance was set at $p<0.05$. All data were analyzed with the SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) statistical software package.

Table 1. Clinical characteristics of 109 patients with heterozygous FH classified with or without CAD

	Total $n=109$	CAD(−) $n=88$	CAD(+) $n=21$	p value
Age (years)	41.9±16.2	39.7±16.7	50.9±10.5	<0.01
Sex (male), n (%)	43 (39.4%)	30 (34.1%)	12 (57.1%)	0.052
Achilles tendon thickness (mm)	12.6±5.4	11.5±4.5	17.4±6.3	<0.0001
Skin xanthomas, n (%)	25 (22.9%)	16 (18.2%)	9 (42.9%)	0.052
Arcus cornea, n (%)	45 (41.3%)	27 (30.7%)	16 (76.2%)	0.001
Total cholesterol (mg/dL)	321±68	309±56	368±92	<0.001
Triglyceride (mg/dL)	139±82	134±85	156±65	0.272
HDL-C (mg/dL)	51±15	51±15	50±15	0.747
LDL-C (mg/dL)	242±70	232±59	287±92	0.001
Smoking (past or current), n (%)	42 (38.5%)	28 (31.8%)	14 (66.6%)	0.003
Hypertension, n (%)	19 (17.4%)	10 (11.4%)	9 (42.9%)	0.003
Diabetes mellitus, n (%)	9 (8.2%)	5 (5.7%)	4 (19.0%)	0.186
Family history of CAD, n (%)	47 (43.1%)	37 (42.0%)	10 (47.6%)	0.411

Table 2. Clinical characteristics at first visit in heterozygous patients of FH classified by LDL-C Levels (Mean ± SD)

LDL-C (mg/dL) categories	LDL-C <206 $n=36$	206≦LDL-C<260 $n=36$	LDL-C ≥260 $n=37$	p value
Age (years)	43.7±15.6	42.0±17.5	40.0±15.8	0.645
Sex (male), n (%)	14 (38.9%)	13 (36.1%)	15 (40.5%)	0.928
Body mass index (kg/m^2)	22.2±3.3	22.5±3.2	22.8±6.8	0.880
Total cholesterol (mg/dL)	258±28	308±20	394±55	<0.001
Triglyceride (mg/dL)	149±102	134±67	134±74	0.672
HDL-C (mg/dL)	54±16	51±15	47±14	0.100
Smoking (past or current), n (%)	15 (41.7%)	10 (27.8%)	15 (40.5%)	0.385
Hypertension, n (%)	5 (13.9%)	6 (16.7%)	3 (8.1%)	0.660
Diabetes mellitus, n (%)	2 (5.6%)	3 (8.3%)	2 (5.4%)	0.831
Family history of CAD, n (%)	17 (47.2%)	14 (38.9%)	16 (43.2%)	0.775
Achilles tendon thickness (mm)	10.7±4.2	12.5±5.5	14.6±5.8	0.282
CAD, n (%)	5 (13.9%)	3 (8.3%)	13 (35.1%)	0.02

Results

Characteristics of the Patients Subjected to Analysis of the Correlations of LDL-C and CAD

Among 109 patients, 21 (19.3%) developed CAD during the subsequent period. There was a significantly higher prevalence of hypertension, skin xanthomas, arcus cornea and smoking in the CAD (+) group. Mean age, ATT, TC and LDL-C were significantly higher in the CAD (+) group than in the CAD (−) group (**Table 1**).

LDL-C Levels and Development of CAD

The clinical characteristics of patients categorized into tertiles according to their LDL-C levels are shown in **Table 2**. They clearly show that parameters other than TC levels were not significantly different in each tertile. Higher LDL-C was associated with higher TC and the incidence of CAD.

To examine the influence of conventional coronary risk factors, logistic regression analyses for CAD were performed. The multivariable adjusted odds ratios (ORs) for CAD are shown in **Table 3**. Individuals in the highest tertile (LDL-C ≥260 mg/dL) had a 8.29-fold increased risk of CAD incidence compared with those in the lowest tertile (LDL-C <206 mg/dL) (adjusted odds ratio (OR) 8.29, 95 % CI 1.33-51.47, $p=0.023$). No significant increase in the odds of future CAD in the second tertile (206≦LDL-C<260 mg/dL) (adjusted OR 0.42, 95%CI 0.05-3.26, $p=0.409$).

Table 3. Multivariate-adjusted odds ratio for CAD by logistic regression analyses according to LDL-C

LCL-C categories	n	Odds Ratio	95% CI	*p* value
LDL-C <206 mg/dL	36	1.0 (referent)	–	–
206≦LDL-C<260 mg/dL	36	0.42	0.05-3.26	0.409
LDL-C ≥260 mg/dL	37	8.29	1.33-51.47	0.023

Multivariable logistic regression models for CAD are adjusted for age, sex, hypertension, diabetes mellitus, smoking, family history of CAD, and low HDL-C (<40 mg/dL).

Table 4. Clinical characteristics at first visit in heterozygous patients of FH classified by ATT levels (mean ± SD)

ATT (mm) categories	ATT <9 *n*=36	9≦ATT<14.5 *n*=37	ATT ≥14.5 *n*=36	*p* value
Age (years)	39.7±18.3	39.4±16.4	45.2±13.5	0.177
Sex (male), n (%)	11 (30.6%)	13 (35.1%)	18 (50.0%)	0.207
BMI (kg/m^2)	22.3±2.8	21.7±2.8	23.1±2.7	0.883
Total cholesterol (mg/dL)	293±42	319±66	350±79	0.002
Triglycerides (mg/dL)	140±106	134±69	142±67	0.505
HDL-C (mg/dL)	57±14	47±14	48±15	0.916
LDL-C (mg/dL)	208±44	245±67	274±78	0.003
Smoking habit, n (%)	9 (25.0%)	13 (35.1%)	14 (38.9%)	0.001
Hypertension, n (%)	4 (11.1%)	2 (5.4%)	8 (22.2%)	0.094
Diabetes mellitus, n (%)	1 (2.8%)	1 (2.7%)	5 (13.9%)	0.125
Family history of CAD, n (%)	16 (44.4%)	17 (46.0%)	14 (38.9%)	0.815
CAD, n (%)	2 (5.6%)	4 (10.8%)	15 (41.7%)	<0.001

ATT Levels and Development of CAD

The clinical characteristics of patients categorized into tertiles according to their ATT levels are shown in **Table 4**. Higher ATT levels were associated with higher TC and LDL-C levels, smoking and the incidence of CAD.

The multivariable adjusted OR for CAD is shown in **Table 5**. Individuals in the highest tertile group of ATT ≥14.5 mm had a 7.82-fold increased risk of CAD compared with those in the ATT <9.0 mm group (95%CI 1.28-47.7, p=0.001). No significant increase in the odds of future CAD in the group with 9≦ATT<14.5 mm (adjusted OR 1.42, 95%CI 0.18-11.14, p=0.740).

LDL-C and/or ATT Levels and Development of CAD

To estimate the future risk for CAD using the combination of LDL-C and ATT thickness, the patients were divided into 3 groups, (1) LDL-C <260 mg/dL and ATT <14.5 mm, (2) LDL-C <260 and ATT ≥14.5, or LDL-C ≥260 and ATT <14.5, (3) LDL-C ≥260 and ATT ≥14.5. OR for CAD was calculated for these groups and shown in **Table 6**. Those who had both LDL-C ≥260 and ATT ≥14.5 had a 20.62-fold increased risk of CAD compared with those with LDL-C <260 and ATT <14.5 (95%CI 2.91-145.89). Those with either LDL-C ≥260 or ATT ≥14.5 had a 23.62-fold increased risk of CAD compared with those with LDL-C <260 and ATT <14.5 (95%CI 3.11-184.16).

Discussion

As the prognosis of heterozygous FH patients varies substantially, the therapeutic regimen should be determined according to the CAD risk of individual patients. High levels of LDL-C and ATT are clinical signs already found at a young age and can be measured easily and non-invasively by family physicians in primary care, so they can be good markers for estimating the future CAD risk of FH. In the present study, we demonstrated the critical levels of LDL-C and ATT for estimation of the CAD risk in Japanese heterozygous patients with FH.

Several studies on the Japanese population have indicated that the serum cholesterol level is correlated significantly with the risk of CAD[16, 17]. The increased CAD incidence seems exponential with the serum cholesterol level in the general population, and it can be

Table 5. Multivariate-adjusted odds ratio for CAD by logistic regression analyses according to ATT levels

ATT (mm) categories	n	Odds Ratio	95% CI	*p* value
ATT < 9 mm	36	1.0 (referent)	–	–
9≦ATT < 14.5 mm	37	1.42	0.18-11.14	0.740
ATT ≥ 14.5 mm	36	7.82	1.28-47.7	0.001

Multivariable logistic regression models for CAD are adjusted for age, sex, hypertension, diabetes mellitus, smoking, family history of CAD and low HDL-C (<40 mg/dL).

Table 6. Multivariate-adjusted odds ratio for CAD by logistic regression analyses according to both ATT and LDL-C levels

LDL-C and ATT categories	n	Odds Ratio	95% CI	*p* value
LDL-C < 260, ATT < 14.5 mm	54	1.0 (referent)	–	–
LDL-C < 260, ATT ≥ 14.5 mm or LDL-C ≥ 260, ATT < 14.5 mm	37	23.94	3.11-184.16	0.002
LDL-C ≥ 260, ATT ≥ 14.5 mm	18	20.62	2.91-145.89	0.002

Multivariable logistic regression models for CAD are adjusted for age, sex, hypertension, diabetes mellitus, smoking, family history of CAD and low HDL-C (<40 mg/dL).

considered low until it hits a certain "threshold". The findings of the relationship with LDL-C levels and the onset of CAD in FH patients seem to show a "right shift" of this profile as LDL-C 2-fold higher and CAD incidence more than 10-fold. Previous studies have reported that higher LDL-C is related with the higher risk factors for the development of CAD even in heterozygous FH patients[18, 19], whereas other factors, such as age, gender, hypertension, smoking, or other lipid abnormalities, such as low HDL-C and high TG reportedly contribute to the increased risk[3, 12, 18-22]. Bujo *et al.* reported that male gender, age over 50, smoking, hypertension, diabetes mellitus, TG >150 mg/dL and HDL-C <40 mg/dL were risk factors for CAD in FH by multicenter, cross-sectional analysis[3].

As we reported in a previous paper, drug treatment including statins may influence the outcome of CAD[5]. The name and dose of drugs prescribed to the patients during the course are listed in **Table 7**. Because all FH patients had had intensive drug therapy to prevent the development of atherosclerosis, no comparison could be made with those without drug therapy. It was also impossible to analyze the difference in drugs statistically because there were so many patterns of prescription and most patients changed the type and dose of drugs several times during the course.

LDL-C levels under drug treatment may also affect the outcome. The mean LDL-C under drug treatment did not increase the odds ratio for CAD (odds ratio: 0.983, 95%CI: 0.97-1.00); however the relationship between mean LDL-C in the pre-treatment period and CAD risk remained due to pre-exposure to high LDL-C before treatment, although the absolute risk of CAD might be decreased at any LDL-C level by intensive drug treatment during the course.

Table 7. Lipid-lowering drugs in heterozygous FH patients during the course

	Dose/day
cholestyramine	4-12 g
colestimide	0.5-3 g
probucol	250-1,000 mg
pravastatin	10-30 mg
simvastatin	5-20 mg
fluvastatin	20-60 mg
atorvastatin	5-40 mg
pitavastatin	1-4 mg
rosuvastatin	2.5-20 mg
fenofibrate	100-200 mg
bezafibrate	100-400 mg
ezetimibe	5-10 mg

Civerira *et al.* reported that heterozygous FH with tendon xanthomas has a 3.1-fold increased risk of premature CAD compared with those without it[23]. The Achilles tendon was reported to be thicker in FH patients with CAD than in those without CAD in both sexes[12]. Persistent high LDL-C causes cholesterol depositions in the tendons and results in tendon xanthomas[1]. Achilles tendon xanthomas have been used

as one of the criteria for clinical diagnosis of FH because of their high sensitivity and specificity[1, 24]. A strongly positive correlation was observed between ATT and cholesterol-year scores in FH patients[25, 26], suggesting that ATT reflects both the severity and duration of hypercholesterolemia. ATT is an important factor that can be measured quantitatively as the deposition of cholesterol in the tissue. The present study showed that ATT is a good marker for evaluating the risk for CAD, indicating that there is a strong correlation between the deposition of cholesterol in extravascular tissue and the stenosis of coronary arteries. ATT should be used not only as a diagnostic parameter for FH but also, and more importantly, as a prognostic factor that indicates the need for a more aggressive approach for patients at high risk.

In conclusion, LDL-C ≥260 mg/dL and ATT ≧14.5 mm or thicker are useful criteria for identifying patients at "very high" risk of CAD in Japanese heterozygous FH. Patients with either of these risk factors require more intensive cholesterol-lowering therapy and a more careful medical check-up for CAD.

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