

## Research Article

# Deposition Patterns and Localization of Apolipoprotein A1 and Their Relation to Plaque Morphology in Human Coronary Artery

Yasumi Uchida<sup>1\*</sup>, Yasuto Uchida<sup>2</sup>, Ei Shimoyama<sup>3</sup>, Nobuyuki Hiruta<sup>4</sup>, Tsuyoshi Tabata<sup>5</sup>, and Takanobu Kobayashi<sup>6</sup>

<sup>1</sup>Japanese Foundation for Cardiovascular Research, Japan

<sup>2</sup>Department of Cardiology, Tsukuba Memorial Hospital, Japan

<sup>3</sup>Department of Pathology, Funabashi-Futawa Hospital, Japan

<sup>4</sup>Department of Pathology, Toho University Medical Center, Japan

<sup>5</sup>Department of Clinical Physiology, Toho University Medical Center Sakura Hospital, Japan

<sup>6</sup>Department of Cardiology, Chia-kensei Hospital, Japan

**\*Corresponding author**

Yasumi Uchida, Japan Foundation for Cardiovascular Research, 2-30-17, Narashinodai, Funabashi, Japan, Tel: 81-47-462-2159; Email: uchida73@ta2.so-net.ne.jp

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- Human coronary plaques
- Intimal vasa vasorum
- Pericoronary adipose tissue

**Abstract**

**Background:** Besides comprising high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1) itself acts as an anti-atherogenic substance but its supplying routes and localization in the human coronary artery wall is poorly understood. The present study was performed to clarify supply route(s) and localization of ApoA1 and its relation to human coronary plaque morphology.

**Methods:** Using 36 coronary arteries obtained from 15 autopsy subjects, deposition patterns of ApoA1 and whether it is conveyed by CD68 (+)-macrophage or vasa vasorum, and whether apoA1 co-localized with HDL were examined immunohistochemically. Also, the relationship between localization of ApoA1 and plaque morphology that was classified by angiography and histology was examined.

**Results:** ApoA1 was deposited in a dotted or diffuse pattern in the coronary plaques. The dotted pattern represented ApoA1 carried by CD68 (+)-macrophages and diffuse pattern by vasa vasorum. The percentage (%) incidence of ApoA1 increased in the order of normal segments, white plaques (growth stage) and yellow plaques (mature stage). ApoA1 was also observed in the pericoronary adipose tissue (PCAT), and ApoA1-containing CD68 (+)-macrophages were observed not only in the intima (plaques) but also in the media, adventitia and PCAT. ApoA1 did not necessarily co-localize with HDL.

**Conclusions:** The findings suggested that ApoA1 was conveyed to coronary plaques either by CD68 (+)-macrophages or vasa vasorum from the adventitia (possibly PCAT) and that ApoA1 began to deposit with plaque growth and increasingly deposited in mature stage of plaques. Accordingly, externally administered ApoA1-mimetics after plaque maturation may not be accepted by the plaques and fail to prevent coronary events. One possible way of making ApoA1-mimetics effective in preventing coronary events, is to administer them before plaque maturation.

**ABBREVIATIONS**

AC: Adipocyte; ApoA1: Apolipoprotein A1; HDL: High-Density Lipoprotein; NC: Necrotic Core; OxLDL: Oxidized Low-Density Lipoprotein; PCAT: Pericoronary Adipose Tissue

**INTRODUCTION**

Besides being a component of high-density lipoprotein (HDL), an important anti-atherogenic substance, apolipoprotein A1 (ApoA1) itself is considered to act against atherosclerosis by mediating reverse cholesterol transport. Among patients treated with statin therapy, an increased plasma level of ApoA1 is associated with reduced cardiovascular risk [1]. Increased ApoB / ApoA1 ratio is associated with endothelial dysfunction

and a risk factor for coronary heart disease [2,3]. In experimental animals, infusions of ApoA1 or its mimetics halt the progression or induce regression of atherosclerosis, with favorable effects on plaque composition [4-6]. In the clinical situation however, it is controversial as to the effects of ApoA1- or HDL-mimetics in improving outcomes for patients with coronary artery disease [7,8].

Previously, we found that oxidized low-density lipoprotein (oxLDL) and HDL are deposited in human pericoronary adipose tissue (PCAT) and the former is supplied by macrophages and neovascularized vasa vasorum and the latter by the vasa vasorum to coronary plaques [9,10], but it is poorly understood whether native ApoA1 is supplied and deposited in coronary plaques

similarly.

The present study was therefore performed to examine the supply route(s) of ApoA1 and the relationship between deposition of ApoA1 and plaque morphology using immunohistochemical techniques [10].

## METHODS

### Angioscopic and immunohistochemical studies of excised human coronary arteries

**Ethics statement:** This *ex vivo* study was carried out after approval of the Ethics Committees of the Japan Foundation for Cardiovascular Research, Funabashi-Futawa Hospital, Tsukuba Memorial Hospital, Sannou Hospital and Toho University, and after obtaining written informed consent from the families concerned on the use of excised coronary arteries and surrounding adipose tissue for angioscopic and histological studies to clarify the mechanisms of atherosclerosis.

**Subjects:** The 36 proximal to middle segments of coronary arteries (12 left anterior descending arteries, 11 left circumflex arteries, 13 right coronary arteries) and the surrounding pericoronary adipose tissue (PCAT) were carefully excised from 15 successive cases of patients who had died and undergone autopsy from April 1, 2014 to June, 2016 at Tsukuba Memorial Hospital, Funabashi-Futawa Hospital, Chiba-kensei Hospital, Sannou Hospital or Toho University Medical Center Sakura Hospital [63.1 ± 9.8 years; 6 females, 9 males; acute myocardial infarction (3), stable angina (1), diabetic nephropathy (3), cerebrovascular disease (3), abdominal carcinoma (4), and sudden death (1)] (Table 1). Coronary arteries were obtained within 5 - 12 h of death, and the examinations were started within 4 - 7h thereafter.

**Classification of coronary plaques and normal segments by conventional angioscopy:** We used a conventional coronary angioscopy system [11,12] to classify coronary plaques and normal segments. Plaque was defined as a non-mobile, protruding or lining mass clearly demarcated from the adjacent normal

wall and with a shape, location and color that did not alter after saline flushing. A normal segment was defined as a milky-white and smooth-surfaced portion without any protrusions [12]. Classification of non-disrupted plaques and normal segments was performed independently using images recorded on DVD disks by two observers who did not participate in the conventional angioscopy. Disrupted plaques were excluded because thrombi and plaque debris might disturb plaque color assessment and immunohistochemical staining.

**Plaque color assessment:** Because color varies from observer to observer, influenced by their experience and visual sense, we developed a more objective method for color definition. Plaque images obtained by conventional angioscopy were classified as white or yellow by an AquaCosmos image analyzer (C7746, Hamamatsu Photonics, Hamamatsu, Japan). Based on the relationship between a color spectrum generated through a prism by a Xenon lamp and its light wavelengths, the light wavelength of "yellow" was defined as between 575 and 595 nm. The color band of the area being imaged through an angioscope was separated by the image analyzer into the three primary colors, namely red, green and blue. The intensity ratio of red: green: blue of the "yellow" color was 1.0: 1.38-1.46: 0.47-0.60. Similarly, white light generated by the Xenon lamp was separated into three primary colors with an intensity ratio of 1.0: 0.9-1.1 : 0.9-1.1, which defined "white" plaque [11-13].

### Observation of excised coronary arteries by conventional angioscopy

**Procedure:** A Y-connector was introduced into the proximal portion of the respective coronary artery for perfusion with saline solution at a rate of 20 mL/min and then the angioscope was introduced through the connector into the artery to evaluate it for plaques. A total of 34 white plaques, 66 yellow plaques and 50 normal segments, which matched the aforementioned criteria were confirmed by conventional angioscopy in the 36 coronary arteries, and all of them were used for the subsequent studies. Externally, the location of each observed plaque or normal

**Table 1:** Backgrounds of Autopsy Subjects.

Patients	Age/gender	Disease	Cause of death	Treatment
1	81/M	Stable angina	Gastric cancer	Nitrates, Clopidogrel, etc
2	80/F	Acute myocardial infarction	Do	Emergency treatment
3	59/M	Acute myocardial infarction	Cerebral bleeding	PCI, nitrates, Diuretics, Clopidogrel
4	55/M	Acute myocardial infarction	CHF	PCI, nitrates, Diuretics, Clopidogrel
5	63/M	Cerebral infarction	Do	Clopidogrel, Silostazol,
6	78/M	Diabetic nephropathy	Colon cancer	Insulin, Hemodialysis
7	72/F	Subarachnoid hemorrhage	Do	Emergency therapy
8	60/M	Gastric cancer	Pneumonia	Gastrectomy
9	52/M	Cerebral infarction	Pneumonia	Antithrombotic therapy,
10	49/F	Diabetic nephropathy	Aortic dissection	Insulin, Hemodialysis
11	67/F	Urinary bladder cancer	Do	Radiation therapy after surgery
12	48/F	Uterus cancer	Do	Radiation
13	63/M	Hepatocellular cancer	Do	Radiotherapy
14	59/F	Sudden death	Do	Emergency therapy
15	60/M	Diabetic nephropathy	Acute myocardial infarction	Insulin, Pitavastatin, etc

Do: the same disease. CHF: congestive heart failure. PCI: percutaneous coronary intervention.

segment could be identified because the light irradiated by the angioscope tip was visible through the arterial wall.

**Selection of plaques and normal segments:** The 4-5-mm long section of artery in which the observed plaque was located and its surrounding PCAT was isolated by transecting its proximal and distal ends along the shorter axes. Normal segments were similarly isolated. Plaques and normal segments that had not been damaged by the manipulation were selected for immunohistochemical analysis. In total, 32 white plaques (1 each from 32 arteries), 66 yellow plaques 1 - 2 each from 33 arteries), and 50 normal segments (1 - 2 each from 33 arteries), surrounded by PCAT were selected, and embedded in O.C.T. Compound (Sakura Finetek USA Inc., Torrance, CA, USA) before being stored at -20°C. The culprit arteries for acute myocardial infarction were excluded.

### Immunohistochemistry of ApoA1, HDL, CD68 (+)-macrophages and CD31

**Definition of PCAT:** The epicardial adipose tissue located within 3 mm (nearly the same as the diameter of the proximal to middle segments of the coronary artery) of the external elastic lamina of an epicardial coronary artery was arbitrarily defined as PCAT because it may more likely to directly influence the coronary artery than epicardial adipose tissue located remotely, based on an observation of micro vessels penetrating from the PCAT to the epicardial coronary arterial wall [14].

**Single immunohistochemical staining:** All plaques and normal segments with their surrounding PCAT, which had been stored at -20°C, were cut into successive 20 µm sections on a cryostat (Tissue Tec 3D, SakuraFinetec Japan, Tokyo). Such relatively thick and frozen sections were used to prevent any substances leaking from the PCAT. Next, the sections were fixed with 4 % paraformaldehyde solution for 7 min at 4°C, and incubated with a mixture of 1% hydrogen peroxide in methanol for 30min. Successive sections were stained immunohistochemically in the order of apoA1, HDL, CD68 for CD68 (+)-macrophages and CD31 for endothelial cells of the vasa vasorum (arising from the mother vessel and/or penetrating microvessels arising from the PCAT). Briefly, a section was reacted with anti-apoA1 antibody (Anti-apoA1-antibody orb 10973; rabbit polyclonal, which reacts with human apoA1; Biorbyt Ltd, Cambridge, UK) diluted 100-fold (µg/mL) for 60 min, the peroxidase reaction was developed by 3, 3'-diaminobenzidine tetrahydrochloride using an Envision kit (Code No K4061, DAKO Co, Grostrup, Denmark) for 30 min, and finally the cell nuclei were stained with hematoxylin. The next section was pretreated similarly and reacted with anti-HDL antibody [1C5] ab 34788 abcam, Mouse monoclonal [1C5] to HDL, the antibody reacts with HDL2 and HDL3, reacts with human HDL (Abcam Ltd, Tokyo, Japan). The third section was reacted with anti-CD68 antibody (mouse monoclonal NCL-CD 68-KP1; antigen lysosomal granules from human lung macrophages; Leica Biosystems Newcastle Ltd, Newcastle, UK) diluted 200-fold, and treated similarly to stain CD68(+)-macrophage [15,16]. The fourth section was reacted with anti-CD31-antibody (rabbit polyclonal; immunogen: synthetic peptide corresponding to C terminals of

mouse CD31; reacts with mouse and human CD31; Abcam Co, Tokyo, Japan) diluted 50-fold, reacted with biotinylated anti-goat IgG diluted to 100-fold, and treated similar to ApoA1 to stain CD31 in the endothelial cells of the vasa vasorum. Using these immunohistochemical techniques, all relevant molecules were stained brown.

**Double immunohistochemical staining:** Double staining of ApoA1 and CD68 was performed to confirm that apoA1-containing mononuclear cells were CD68 (+)-macrophages. Briefly, after washing with phosphate-buffered saline, a section was reacted with anti-CD68 antibody as described before for 60 min, and with anti-mouse Alexa555 (Alexa Fluoro555 goat anti-mouse IgG, Code A21422, Molecular Probe Ltd, CA, USA) for 30 min to elicit the red fluorescence of CD68. The same section was reacted with anti-apoA1 antibody for 60 min, and with anti-rabbit FITC (FITC conjugated AffeiniPureoat Anti-Rabbit IgG, Code 111-095-003, Vector Laboratories Inc, Burlingame, CA, USA) for 30 min to elicit the green fluorescence of apoA1. Finally, the section was reacted with DAPI (4', 6-diamidino-2-phenylindole; Life Technologies Carlsbad, Carlsbad, CA, USA) to elicit the blue fluorescence of cell nuclei [17]. ApoA1, macrophages and their nuclei thus stained were photographed separately or merged with one another through a confocal laser scanning microscope (FLUOVIEW FV 1700; Olympus Co, Tokyo, Japan) using a 460 nm band-pass filter (BPF) and a 510 nm band absorption filter (BAF) to obtain the green fluorescence of ApoA1, a 555 nm BPF and 575 nm BAF to obtain the red fluorescence of macrophages, and a 345 nm BPF and 420 nm BAF to obtain the blue fluorescence of cell nuclei.

### Microscopic observation of the coronary artery after immunohistochemical staining

**Definition of the deposition pattern of ApoA1:** Histological observation of the deposition of ApoA1 was performed using a microscope (IX 70, Olympus Co.). The deposition pattern classified as dotted (i.e. diameter ≤ 30 µm) or diffuse (diameter ≥ 100 µm). We considered the dotted deposition as significant if the dot density was ≥ 5 /200 × 200 µm<sup>2</sup>, because a small number of dots were disseminated not only in the intima but also in the PCAT in the majority of sections stained for ApoA1.

**Relationship between plaque morphology and ApoA1 deposition:** The percentage (%) incidence of ApoA1 deposits (both dotted and diffuse) in the PCAT, adventitia, and intima was compared among normal coronary segments, white plaques and yellow plaques with and without necrotic core (NC). Deposition in the intima (including plaque) was further classified as inner layer (luminal side) or outer layer (medial side) deposition.

**Relationship between intimal vasa vasorum and ApoA1 deposition:** By staining with CD31, a marker of vascular endothelial cells, we examined whether the deposition of ApoA1 in the intima was dependent on the formation of intimal vasa vasorum, which penetrates from the adventitia into the intima.

### STATISTICAL ANALYSIS

The data obtained were tested by Fisher's exact test or

Mann-Whitney's U-test. A value of  $p < 0.05$  was considered to be statistically significant.

## RESULTS

### Deposition pattern of ApoA1

ApoA1 deposited in the coronary intima in either a dotted (Figure 1A-1) or diffuse (Figure 1B-1) pattern. In the adventitia and media, ApoA1 deposited in a dotted pattern only (Figure 1A-1). On magnification, the dotted ApoA1 deposits were round (Figure 2A,D) or spindle-like in shape (Figure 2B,C).

### Relationship between deposition of ApoA1 and plaque morphology

The percentage (%) incidence of ApoA1 (both dotted and diffuse patterns) in the intima was low in normal segments, increased in white plaques (growth stage) and yellow plaques without NC (NC; mature stage) and further increased in yellow plaques with NC (end-stage of maturation) as classified by conventional angiography and histology (Table 2). The NC was filled with ApoA1 in all preparations examined (Figure 3).

The % incidence of dotted ApoA1 deposits was highest in normal segments, showed a tendency to decrease in white plaques, and significantly decreased in yellow plaques, whereas that of diffuse ApoA1 deposits was low in normal segments and increased in yellow plaques (Table 3).

### Deposition of ApoA1 in the PCAT

ApoA1 was stained in all PCAT specimens in either the dotted (Figure 1A-2,B-2) or diffuse pattern (Figure 1A-3, B-3). The

former was found in the interstitial space between adipocytes (Figure 1A-2,B-2) and the latter in the cytoplasm and plasma membrane of the adipocytes that comprised the PCAT (Figure 1A-3,B-3).

### Dotted ApoA1 deposits were that contained in CD68 (+) - macrophages

Double immunohistochemical staining of ApoA1 and macrophages revealed that dotted ApoA1 was contained in CD68 (+)-macrophages in both in plaques and PCAT (Figure 4). In the PCAT, the CD 68(+) -macrophages were observed in the interstitial space between adipocytes (Figure 4B,B-2).

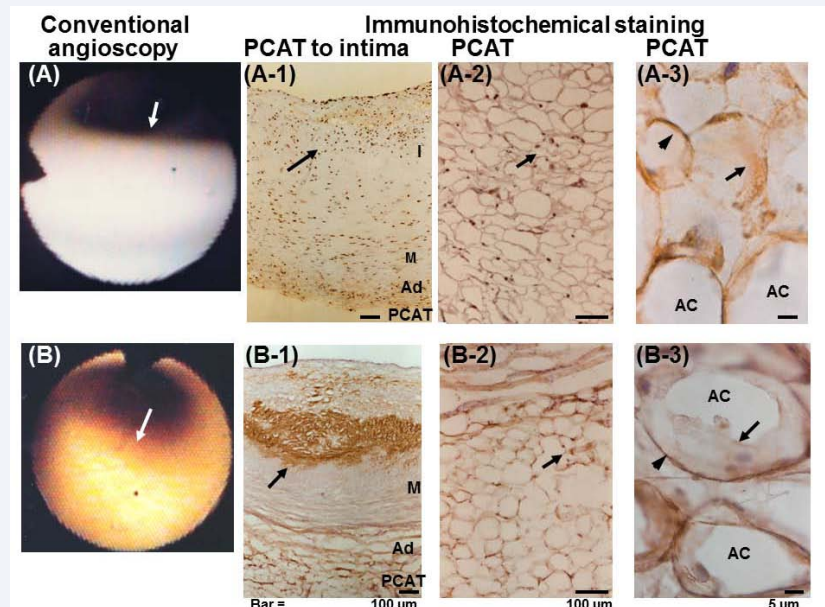
### Co-localization of diffuse ApoA1 deposits and intimal vasa vasorum

Localization of ApoA1 and intimal vasa vasorum (stained by anti-CD31 antibody) was compared in adjacent sections. Diffuse ApoA1 deposits co-localized with vasa vasorum, whereas dotted ApoA1 deposits did not (Figure 5)(Table 4).

Diffuse ApoA1 deposits were co-localized with vasa vasorum in the outer half (medial side) or in both the inner (luminal side) and outer halves (medial side) of the intima in the majority of preparations (Figure 5)(Table 5).

### Co-deposition of ApoA1 and HDL

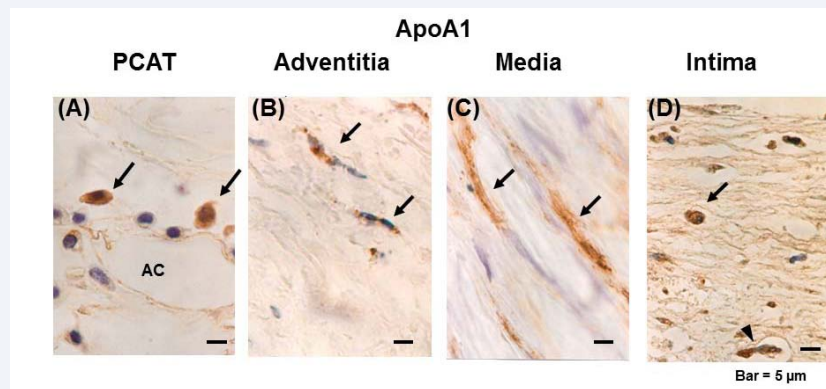
ApoA1 was co-deposited with HDL (Figure 6A,A-1), or they deposited solitarily (Figure 6B,B-1). They did not necessarily co-deposit in normal segments, white plaques and yellow plaques without NC and co-deposited in all yellow plaques with NC. The incidence of both increased with plaque growth and further with



**Figure 1** Deposition Patterns of Apolipoprotein A1 (ApoA1) in Coronary Plaques and Surrounding Pericoronary Adipose Tissue (PCAT).

Immunohistochemical staining of the transected surface of a white plaque (arrow in A) shows dotted ApoA1 deposits in the intima (arrows), media, adventitia (black arrowhead in A-1), and PCAT (arrow in A-2).

Immunohistochemical staining of the transected surface of a yellow plaque (arrow in B) shows diffuse ApoA1 deposits (arrow in B-1), and dotted ApoA1 deposits in the PCAT (arrow in B-2). Cytoplasm (arrows in A-3, B-3) and plasma membrane (arrowheads in A-3, B-3) of the adipocytes (AC) contain ApoA1. I: intima. M: media. Ad: adventitia. Bar in A-1, A-2, B-1, B-2 = 100  $\mu$ m. Bar in A-3, B-3 = 5  $\mu$ m.



**Figure 2** Configuration of Dotted Apolipoprotein A1 (ApoA1).

ApoA1 deposits appear in a round or spindle-like configuration in the pericoronary adipose tissue (PCAT) (arrows in A), adventitia (arrows in B) and the intima (arrow and arrowhead in D), but always appear spindle-like in the media (arrows in C). Bar = 5 µm.

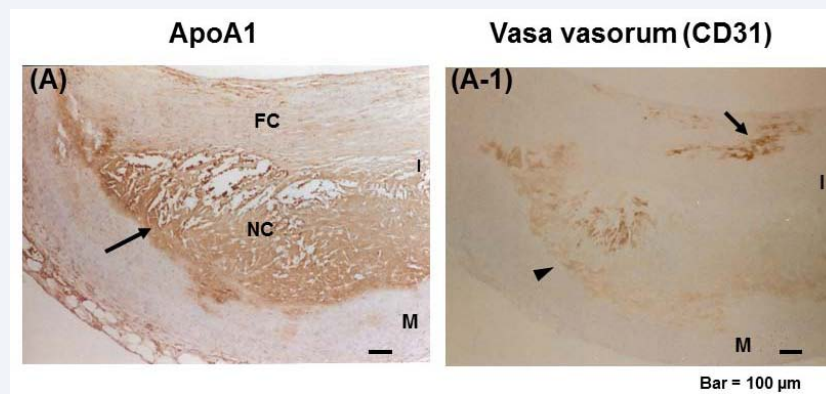
**Table 2:** Relationship between Incidence of Apolipoprotein A1 (ApoA1) and Plaque Morphology.

	Normal	segments	White plaques	Yellow plaques
			NC(-)	NC(+)
n =	50	32	34	32
ApoA1	8	20**	28***	32****
(%)	(16.0)	(62.5)	(82.3)	(100)
HDL	12	15	23 ††	32 †††
(%)	(24.0)	(46.8)	(70.7)	(100)

n= number of preparations examined.

\*\**p*<0.01, \*\*\**p*<0.001, \*\*\*\**p*<0.0001 vs. Normal segments. ††: *p*<0.01, †††: *p*<0.001 vs. Normal segments. NC: necrotic core.

ApoA1 and HDL began to deposit in the coronary plaques at growth stage and increasingly deposited in mature plaques, but they did not necessarily co-deposit.



**Figure 3** Deposition of Apolipoprotein A1 (ApoA1) in Necrotic Core (NC)

A: Necrotic core (NC) filled with ApoA1 (arrow). Vasa vasorum penetrating from the fibrous cap (FC) and media (arrowhead) into the NC. I and M: intima and media, respectively. Bar = 100 µm.

plaque maturation (Table 2).

## DISCUSSION

### CD68 (+) -macrophages is a carrier of ApoA1 to the plaques

In the present study, ApoA1 deposited in the adipocytes of PCAT and ApoA1-containing CD 68(+) -macrophages were observed not only in the adventitia, media and intima but also

in the PCAT, and were also frequently observed traversing the adventitia and media. These findings suggested that CD68 (+)-macrophages obtained ApoA1 from the PCAT, and then conveyed it to the intima. Because there are many phenotypes of macrophages [15], it remains to be examined which phenotype conveys ApoA1.

### Vasa vasorum is a supply route for ApoA1

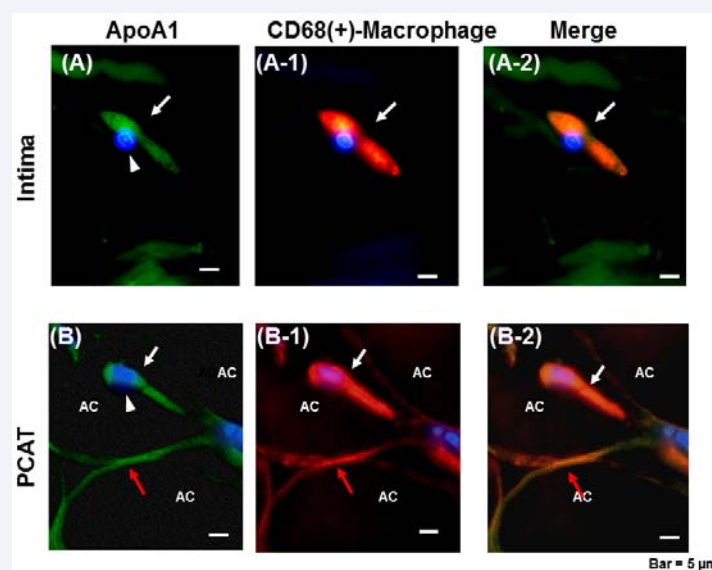
ApoA1 also frequently deposited diffusely in the outer half,

**Table 3:** Relationship between Deposition Patterns of Apolipoprotein A1 and Plaque Morphology.

	Normal	segments	White plaques	Yellow plaques
	(a)	(b)	(c)	(d)
n=	8	20	28	32
Deposition patterns				
(A) Dotted	8**††††	13‡‡§§§§	3	0
(%)	(100)	(65.0)	(10.7)	(0.0)
(B) Diffuse	0	7	13	28
(%)	(0.0)	(35.0)	(40.3)	(87.5)
(C) Both	0	0	12##φ	4
(%)	(0.0)	(0.0)	(42.8)	(12.5)

n= number of preparations examined. NC: necrotic core. \*\*: p<0.01 vs. (c). ††††: p<0.0001 vs. (d). ‡‡: p<0.01 vs. (c). §§§§: p<0.0001 vs. (d). ||: p<0.05 vs. (a). ##: p<0.01 vs. (b). φ: p<0.05 vs. (d). NC: necrotic core.

The incidence of dotted ApoA1 deposits was highest in normal segments and decreased with plaque maturation, whereas that of diffuse ApoA1 deposits increased with plaque maturation.



**Figure 4** Double Immunohistochemical Staining of Apolipoprotein A1 (ApoA1) and CD68 (+) -Macrophages  
CD68 (+) -macrophage containing ApoA1 in intima (arrows in A - A-2) and pericoronary adipose tissue (PCAT) (white arrows in B - B-2). Arrowheads in A and B: nuclei. Red arrows: plasma membrane. Bar = 5 μm.

**Table 4:** Relationship between Deposition Pattern of Apolipoprotein A1 (ApoA1) and Presence or Absence of Intimal Vasa Vasorum.

	Dotted	Diffuse	Both
n=	24	48	16
(a) Vasa vasorum			
Observed (%)	5 (21)*	36 (75)††	15 (94)‡‡
Not observed (%)	19 (79)	12 (25)	1 (6)

n= number of preparations examined.

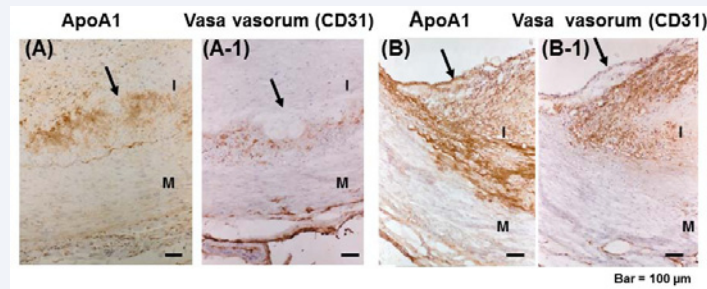
\*: p<0.05, ††: p<0.01, ‡‡ p<0.01 vs. vasa vasorum not observed group.

Dotted ApoA1 deposits were more frequently observed in the vasa vasorum not observed group than in the observed group and vice versa for diffuse ApoA1.

namely the medial side, of plaques and co-localized with the vasa vasorum (probably neovascularized) in white plaques (growth stage) but was distributed throughout the entire yellow plaques (mature stage), suggesting that ApoA1 was conveyed by the vasa vasorum from the adventitial side (possibly from the PCAT) to these locations.

**PCAT is a storage site of ApoA1**

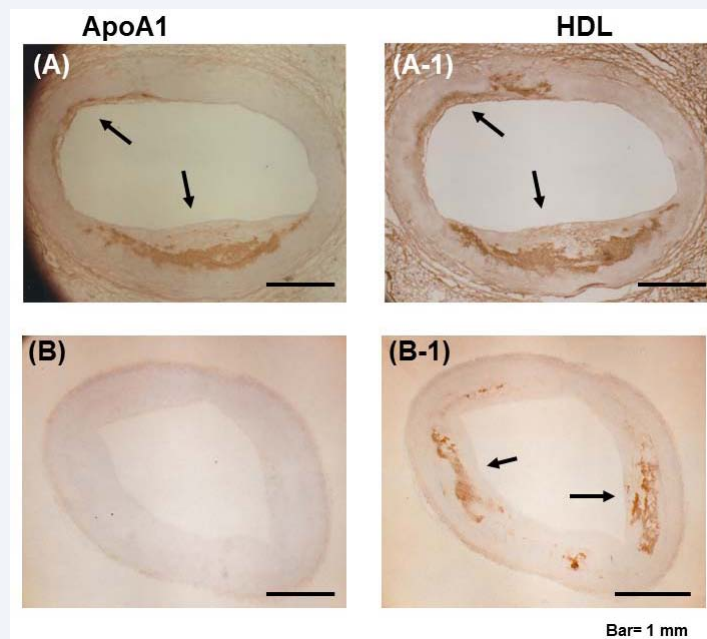
Perivascular adipose tissue, such as PCAT also produces large numbers of metabolically active substances with both endocrine and paracrine actions that can induce or inhibit atherosclerosis [18]. To date, however, it was not known that PCAT stores and supplies ApoA1 to the adjacent coronary arterial intima, which is the site of atherosclerosis as with other perivascular adipose tissue. In the present study, ApoA1 deposition in the PCAT (both adipocytes and plasma membrane) was observed in all



**Figure 5** Relationship between Apolipoprotein A1 (ApoA1) Deposition and Localization of the Intimal Vasa Vasorum.

Diffuse ApoA1 deposits and vasa vasorum co-localize in either the outer layer (medial side) of the plaque (arrows in A and A-1), or in the entire plaque (arrows in B and B-1). Bar = 100 μm.

I: intima. M: media.



**Figure 6** Co-deposition of Apolipoprotein A1 (ApoA1) and High-density Lipoprotein (HDL)

ApoA1 and HDL were co-deposited in the same portions of a plaque (arrows in A and A-1). In other plaque, HDL deposited but ApoA1 not (B and B-1).

**Table 5:** Co-localization of Diffuse Apolipoprotein A1 (ApoA1) Deposits and Intimal Vasa Vasorum.

	Inner half	Outer half	Both
n =	3	17	31
Localization of diffuse ApoA1			
(A) Inner half (%)	2(66)	0 (0)	2 (6)
(B) Outer half (%)	0 (0)	14 (82)**†	4 (13)
(C) Both halves (%)	1 (33)	3 (18)	25 (81)####§§§

n= number of preparations examined.

\*\**p*<0.01 vs. Inner half, †*p*<0.05 vs. both halves, ####*p*<0.0001 vs. inner half, §§§*p*<0.001 vs. outer half.

When the vasa vasorum localized in the outer half of the intima (medial side), ApoA1 deposits also localized to the same half, and when the vasa vasorum was distributed in both halves, ApoA1 deposits were also distributed in both.

cases, irrespective of the presence or absence of atherosclerotic plaques in the adjacent coronary artery segment or ischemic heart disease as the underlying disease as in the case of oxLDL and HDL [9]. However, it remains to be elucidated whether ApoA1 is synthesized in the PCAT or supplied from outside, via the adventitial vasa vasorum or penetrating microvessels that

directly connect the myocardium with the PCAT.

### ApoA1 deposits in mature plaques

The incidence of ApoA1 was low in normal coronary segments, increased in white plaques and yellow plaques

without NC and further increased in yellow plaques with NC, indicating that ApoA1 begins to deposit before plaque formation, increasingly deposits with plaque growth and further increases in the plaque's mature stage.

The NC was filled with ApoA1 in all yellow plaques with NC. Vasa vasorum was observed in the bottom (medial side) of the NC or in the fibrous cap. Such neovascularized vasa vasorum might have transported the ApoA1 into the NC. Whether or not the ApoA1 in the NC protected the plaques from becoming vulnerable, or the ApoA1 oxidized [19-21] and lost its anti-atherogenic action, remains to be elucidated.

### Comparison of deposition pattern and supply route between ApoA1, HDL and OxLDL

Previously, we reported that HDL deposits in human coronary plaques in diffuse pattern while oxLDL in either dotted or diffuse pattern, that HDL is conveyed by vasa vasorum alone whereas oxLDL either by CD68 (+)-macrophages or vasa vasorum, that HDL increasingly deposits with plaque maturation whereas oxLDL increases at growth stage and decreases with plaque maturation, and that both HDL and oxLDL co-deposit in PCAT [9]. Thus, ApoA1 resembles oxLDL in deposition pattern in the plaques and supply routes while resembles HDL in its relation to plaque morphology.

### CLINICAL IMPLICATIONS

In clinical situation, the inhibitory effect of ApoA1- or HDL-mimetics on atherosclerosis is inconsistent [22]. The present study revealed that native ApoA1 was filled in mature plaques, and accordingly, externally administered ApoA1-mimetics may not be accepted by mature plaque and fail to prevent plaques from becoming vulnerable. One possible way of making ApoA1-mimetics effective in preventing coronary events, is to administer them before plaque maturation. Acceleration of ApoA1 deposition in PCAT would be another approach for atherosclerosis prevention.

### CONCLUSIONS

Using immunohistochemical techniques, localization of ApoA1 and its relation to plaque morphology were investigated in coronary arteries obtained from autopsy subjects, and the following findings were obtained. (1) Apo A1 deposited in dotted or diffuse pattern in human coronary plaques. (2) Dotted ApoA1 is contained in CD68 (+)-macrophages and diffuse ApoA1 co-existed with vasa vasorum. (3) ApoA1 began to deposit with plaque growth and increased further with plaque maturation. (4) ApoA1 did not necessarily co-deposit with HDL.

### STUDY LIMITATIONS

1. Because the majority of the study patients were admitted in serious terminal stage, plasma ApoA1 levels examined may not reflect levels under stable clinical conditions.

2. Because the number of patients was small, the differences in the incidence and deposition patterns of ApoA1 could not be compared between the underlying diseases.

3. Oxidation of ApoA1 is known evidence, and therefore it remains to be elucidated whether or not the ApoA1 in mature

coronary plaques, especially in the NC, is the oxidized form.

### AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: Yasumi Uchida and Yasuto Uchida. Performed the autopsy and immunostaining: E.S, N.H. Performed angiography: Yasumi Uchida, Yasuto Uchida, M.K., T.K. Performed statistical analysis: T.T. Wrote the manuscript: Yasumi Uchida.

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