

## DISEASES DISCOVERED IN JAPAN AND THE ROLE OF NUCLEAR CARDIOLOGY —REVIEW ARTICLE

### Imaging Modalities for Triglyceride Deposit Cardiomyovasculopathy

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Received: June 16, 2017/Revised manuscript received: July 13, 2017/Accepted: July 14, 2017

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#### Abstract

Triglyceride deposit cardiomyovasculopathy (TGCV) is a novel clinical entity we found in patients with severe heart failure waiting for cardiac transplantation. The probands carried genetic mutations in adipose triglyceride lipase (ATGL), which is an essential molecule for the intracellular hydrolysis of TG. Patients with TGCV showed ectopic accumulation of TG in cardiomyocytes and smooth muscle cells resulting from the abnormal intracellular metabolism of TG and its substrates, long chain fatty acids (LCFAs). Our postmortem analyses demonstrated substantial numbers of autopsied individuals with TGCV phenotype, whose expression of ATGL was conserved. These results suggest that TGCV might be difficult to diagnose in daily clinics, however be more prevalent than expected, and a possible important cause of cardiac death. This review article focuses on imaging modalities to evaluate cardiomyovascular TG accumulation of this novel disease. Data and images are shown from pathological analyses, imaging mass spectrometry, myocardial scintigraphy with a radioactive analogue for LCFA, iodine-123- $\beta$ -methyl iodophenyl-pentadecanoic acid, MR spectroscopy, and CT-based TG imagings in primary TGCV patients with genetic ATGL deficiency, which is ultra-rare, but the monogenic model.

**Keywords:** Adipose triglyceride lipase, CT-based TG imaging, Imaging mass spectrometry, Iodine-123- $\beta$ -methyl iodophenyl-pentadecanoic acid, MR spectroscopy, Pathological analyses, Triglyceride deposit cardiomyovasculopathy

Ann Nucl Cardiol 2017 ; 3 (1) : 000-000

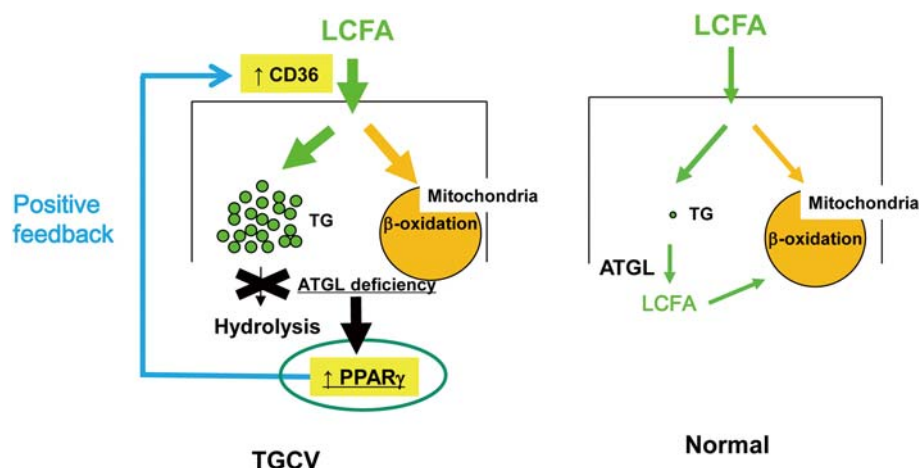
**T**riglyceride deposit cardiomyovasculopathy (TGCV) is a novel clinical entity we found in Japanese patients waiting for heart transplant in 2008 (1, 2). In TGCV, abnormal intracellular metabolism renders cells incapable of using long-chain fatty acids (LCFAs) (3, 4) (Fig. 1), which play major roles in producing ATP in normal heart. TGCV is characterized by the ectopic accumulation of TG in car-

diomyocytes (5) and vascular smooth muscle cells (SMCs) (6) (Fig. 2) that in turn causes pathological conditions such as severe heart failure, coronary artery disease, and arrhythmias. Tissue TG contents in TGCV are not always correlated with BMI, body weight, and obesity which reflect the degree of TG accumulation in adipose tissues, which store TG in physiological conditions. In addition, it has no direct relationship with

doi: 10.17996/anc.17-00028

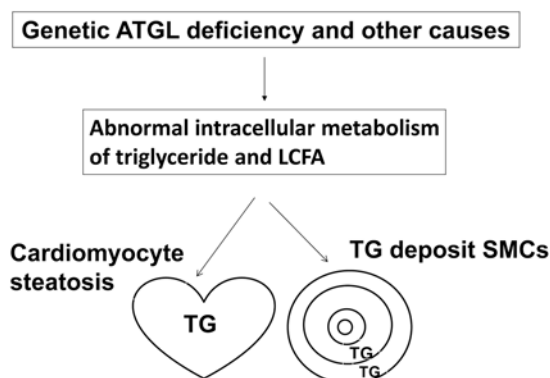
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**Fig. 1** A pathophysiological model for TGCV. ATGL mutations and other causes result in abnormal intracellular metabolism of TG and LCFA, leading to cardiomyocyte steatosis and TG-deposit SMCs. In normal condition (right panel in Fig. 2), LCFA is taken up through LCFA transporters and receptors such as CD36 and some of them are transported to mitochondria for  $\beta$ -oxidation and the remaining LCFAs are utilized as a source for TG and rapidly hydrolyzed by intracellular lipases such as ATGL. In the ATGL mutants, LCFAs are taken up and used for the synthesis of TG which can not be hydrolyzed, leading to massive TG accumulation. Particularly in human ATGL deficiency, PPAR $\gamma$  and related genes are up-regulated, which induces positive feedback mechanism by which the uptake of LCFAs are increased despite the massive deposition of TG.

### Triglyceride deposit cardiomyovascuopathy



**Fig. 2** A schematic presentation of the disease concept for triglyceride deposit cardiomyovascuopathy.

plasma TG levels, because regulatory mechanism and responsible enzymes are different intracellularly from those in plasma (7). The probands for TGCV carried genetic mutations in the adipose triglyceride lipase (ATGL), which is the rate-limiting enzyme for hydrolysis of intracellular TG (8). Gene targeting in mice showed massive cardiomyocyte steatosis and premature death (9). Human genetic ATGL deficiency (10, 11) has turned out to be ultra-rare (12) and only 47 patients identified globally, since we launched the Japan TGCV study group in 2009. However, our postmortem analyses (13, 14) for subjects who died of cardiovascular diseases demonstrated that substantial numbers of autopsied individuals showed TGCV phenotype, most of who had suffered from diabetes mellitus. The amount of triglycerides in the coronary artery of

patients who died of cardiovascular complications was several times higher in diabetic patients than in non-diabetic patients. In addition, we reported cardiomyocyte steatosis and reduced expression of myocardial ATGL in a diabetic model, db/db mouse (15). Based upon these observations, we think that TGCV might be difficult to diagnose in daily clinics, however be more prevalent than expected, and a possible important cause of cardiac death. This review article focuses on imaging modalities which are useful for the diagnosis of TGCV and discuss about future perspectives.

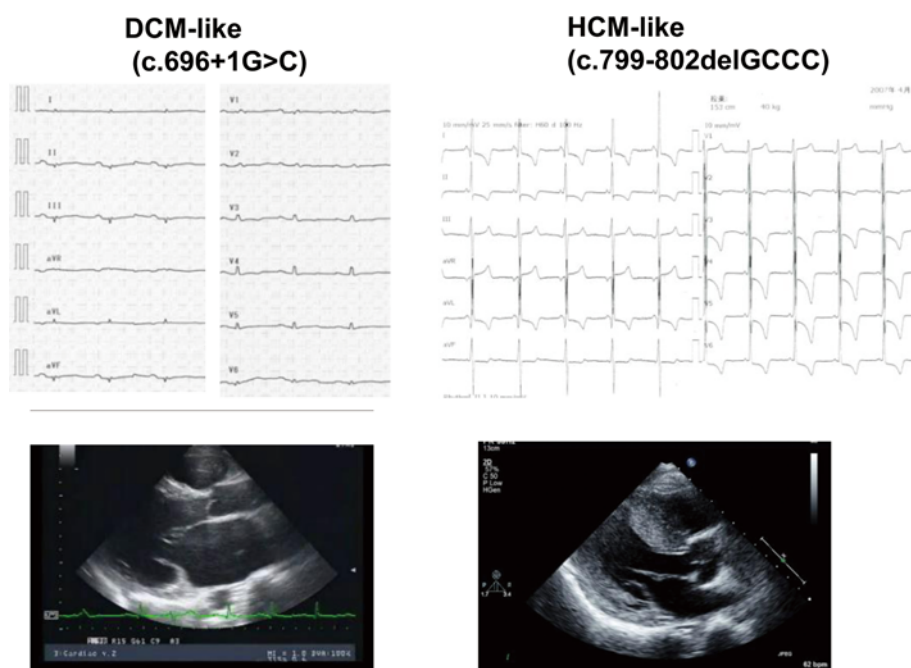
### Classification and epidemiology of TGCV

#### 1. Primary TGCV

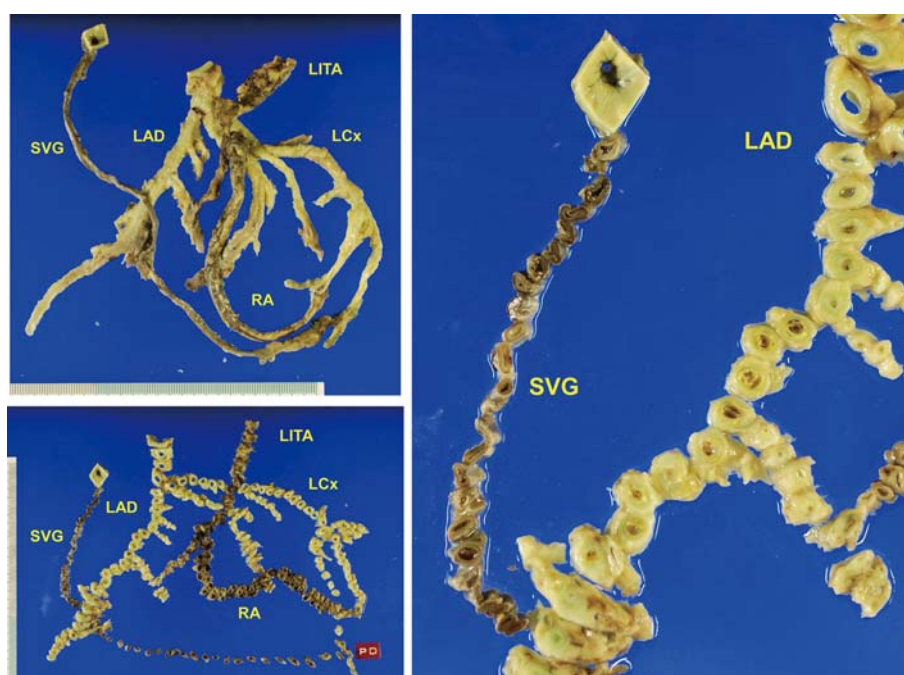
The currently known genetic cause of TGCV is mutations in the ATGL. Homozygotes develop TGCV. Seven homozygous cases have been identified in Japan (2 living, 5 dead) and 47 have been reported globally. Primary TGCV patients have diverse phenotypes that resemble conditions such as dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy (HCM) (16) (Fig. 3). ATGL deficiency manifesting skeletal muscle myopathy (mild to severe) is called neutral lipid storage disease with myopathy (NLSD-M) (10, 11). The phenotype of heterozygotes is currently unknown, and our study group is conducting research on that topic.

#### 2. Idiopathic TGCV

This type of TGCV was found by retrospective postmortem analysis of hearts autopsied after cardiac death (Fig. 4) (13, 14). As with primary TGCV, patients with idiopathic TGCV also exhibit triglyceride accumulation in cardiac muscle and



**Fig. 3** ECG (upper panels) and UCG (lower panels) in ATGL-deficient patients with DCM-like (left panels) and HCM-like (right panels) cardiac morphology. Genetic mutations, clinical profiles, and cardiac phenotype were reported as Cases 2 and 7 in our previous literature.



**Fig. 4** Retrospective postmortem examination revealed a 38-year-old male with TGCV phenotype who died of cardiac attack. He started complaining chest pain at his 30s, followed by coronary artery bypass surgery. Diffuse narrowing stenosis in multi-vessels are shown. The expression of ATGL was conserved (data not shown).

coronary SMCs. Unlike patients with primary TGCV, patients with idiopathic TGCV are not ATGL gene-deficient. The genetic cause of this type is currently unknown, and it is estimated to affect 40,000 to 50,000 people in Japan. Diabetes mellitus is a common complication among patients with primary TGCV as well as those with idiopathic TGCV.

### Disease concept and pathophysiology for TGCV

#### 1. Molecular mechanisms at the cellular level (Fig. 1 and 2)

Normal cardiomyocytes take up LCFAs through transporters such as CD36 and use them as a primary energy source. Once LCFAs enter into a cell, they follow one of two pathways: they are either transported directly to the

**Table 1** Comparison between Cholesterol- and TG-deposit atherosclerosis

	Cholesterol-deposit	TG-deposit
Lesion	Focal	Diffuse
Stenotic type	Eccentric	Concentric
Foam cells, mainly	Macrophages	Smooth muscle cells
Vessel layer involved	Intima	All layers
Underlying mechanism	Response to injury Scavenger receptor	Abnormal intracellular TG metabolism

mitochondria and undergo  $\beta$ -oxidation to produce ATP, or temporarily become triglycerides, are promptly hydrolyzed by an enzyme such as ATGL and transported to the mitochondria to undergo  $\beta$ -oxidation. Patients with TGCV have abnormalities in ATGL and other components of the intracellular triglyceride hydrolysis pathway that cause intracellular accumulation of triglycerides as lipid droplets (3, 4). Lipotoxicity from intracellular triglyceride accumulation and energy failure caused by the inability of cells to use this energy source are involved in the pathogenesis of TGCV.

## 2. Cardiac morphology

As we reviewed literatures, 38% of patients with genetic deficiency showed symptomatic cardiac involvements (12). We reported that even homozygous siblings with the same mutation showed distinct cardiac phenotype; one showed DCM-like and the other showed HCM-like phenotypes (16).

## 3. Novel type of atherosclerosis with TG-deposit smooth muscle cells

The nomenclature for TGCV was derived from the finding of a new type of atherosclerosis characterized by triglyceride accumulation, but not cholesterol. In usual cholesterol-induced atherosclerosis, oxidized low density lipoproteins are taken up through scavenger receptors in macrophages, leading to the formation of eccentric and focal stenosis. In contrast, patients with TGCV exhibit atherosclerosis characterized by “accumulation of triglycerides in vascular SMCs that produces concentric and diffuse stenosis,” which is a unique feature of this disease (2, 6), as summarized in Table 1. The mechanisms underlying the development and progression of this type of atherosclerosis are still unknown. However, *in vitro* studies have shown that vascular endothelial cells (17) and SMCs (18) with TG accumulation have pro-inflammatory and vulnerable characteristics, including increased NF- $\kappa$ B signaling, increased expression of interleukin-6, TNF $\alpha$ , and decreased expression of collagen type I.

## Conceptual difference between TGCV and other diabetic and metabolic heart diseases

There have been known some disease concepts which need to be differentiated from TGCV: one is diabetic cardiomyopathy which was originally defined as cardiomyopathy

without significant stenosis in epicardial coronary arteries. Another is fat accumulation in the epicardial adipose tissue, which is the over-deposition of TG in physiological tissue. TGCV is distinct from these two entities, because TGCV is characterized by the ectopic deposition of TG in cardiomyovascular systems with apparent involvement of epicardial coronary arteries.

## Clinical symptoms for TGCV

The onset of TGCV is believed to appear in the adulthood and middle age. Patients complain of heart failure symptoms such as palpitations, shortness of breath, dyspnea on exertion, fatigability, edema, cough, weight gain, and frequent urination (particularly at night). The chest pain and chest tightness observed in patients with TGCV also occurs at rest and at night, and frequently is resistant to nitroglycerin tablets or nitrates. In some cases, arrhythmia causes symptoms such as pulse deficit, palpitations, and syncope. Many TGCV patients have a history of cardiopulmonary arrest, and there have also been reports of sudden death (16).

## Laboratory tests for TGCV

The pathology of TGCV is rooted in abnormal cellular metabolism, which means that abnormalities in metabolism of TG and LCFAs in affected organs and cells must be evaluated by testing.

### 1. Triglyceride accumulation in peripheral polynuclear leukocytes

Vacuolar degeneration (called Jordans' anomaly) in polymorphonuclear leukocytes can be observed on blood smears (May Giemsa staining) (19, 20). These vacuoles are positive for lipid staining such as oil red O.

### 2. Electrocardiograms (ECG)

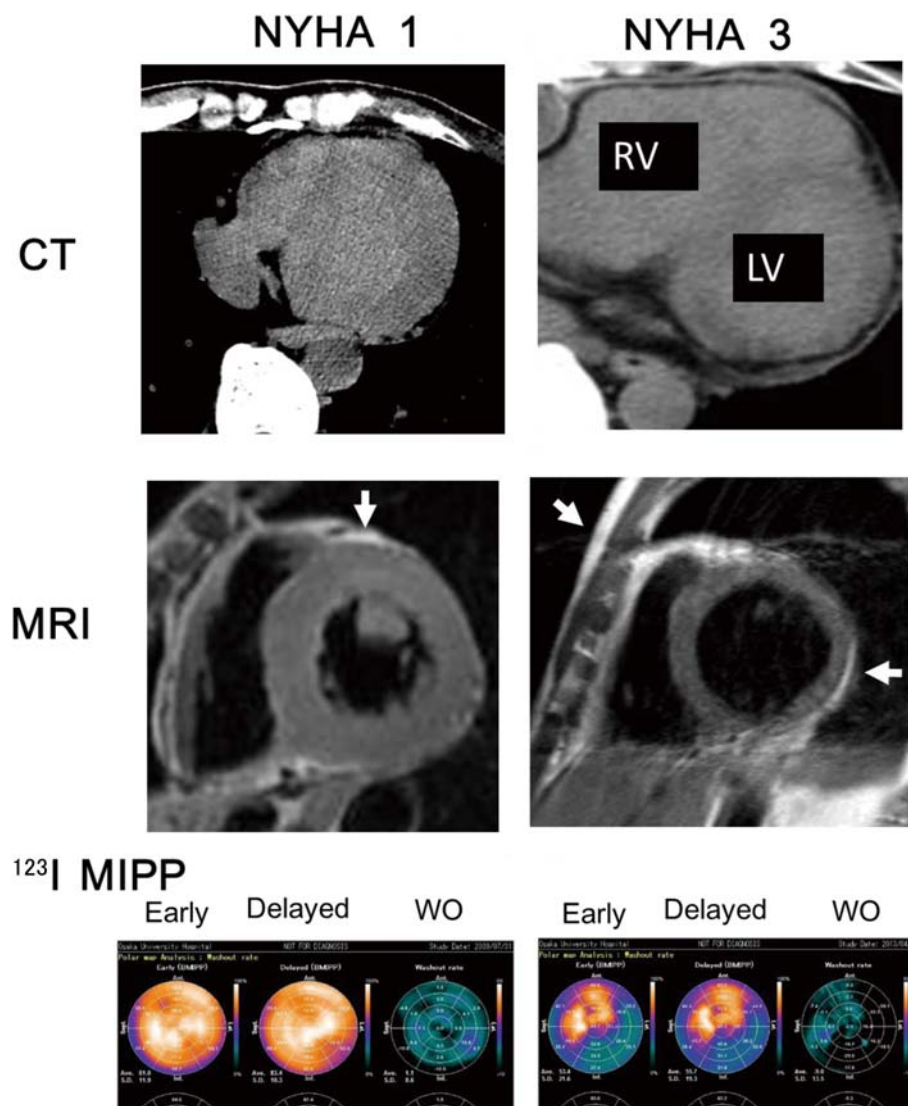
As shown in Fig. 3, ECG reflects cardiac morphology and coronary involvement. There have been no specific findings for cardiomyovascular TG accumulation known.

## Imaging modalities for evaluating myocardial TG accumulation

### 1. Ultrasonography

As shown in Fig. 3, some patients showed DCM-like and





**Fig. 5** CT scan (upper panels), MRI (middle panels), and Polar display of  $^{123}\text{I}$  BMIPP SPECT (lower panels) in patients with genetic ATGL deficiency. The patient in the left panels homozygous for c. 475\_478dupCTCC showed no cardiac symptom (NYHA 1), whereas the right one homozygous for c.576delC suffered from severe heart failure (NYHA 3). White arrows indicate adipose tissue (epicardial or subcutaneous). WO: wash out

others showed HCM-like phenotype. There have been no specific findings in ultrasoundcardiogram for cardiomyovascular TG accumulation known.

## 2. CT scan and MRI

As shown in Fig. 5, myocardial TG accumulation can be detected as black area (low CT value) in CT at the early and progressive stage of the disease. If color-coded display is applied, the TG accumulation can be enhanced (16). MRI can detect adipose tissues (*i.e.* subcutaneous and epicardial fat) (white arrows) in the middle panel of Fig. 5, however even at the progressive stage, myocardial TG deposition is hardly seen. As reported in previous literatures, MR spectroscopy can detect the TG accumulation at the earlier stage of disease (21, 22).

## 3. Myocardial fatty acid imaging with iodine-123- $\beta$ -methyl-iodophenyl-pentadecanoic acid (BMIPP)

BMIPP is a radioactive analogue for LCFA, which are widely used in clinics to image myocardial LCFA metabolism. In normal subjects, BMIPP is rapidly accumulated into myocardium through CD36, trapped in the TG pool, and then metabolites are gradually washout out from the cells due to mitochondrial  $\beta$ -oxidation. Patients with TGCV have a markedly decreased washout rate on BMIPP (lower panels of Fig. 5 and 6) (5).

## 4. Pathological examination with imaging mass spectrometry (IMS)

Myocardial specimens from endomyocardial biopsy, excision from explanted heart at cardiac transplantation and autopsied ones can be used for pathological analyses. Routine hematoxylin and eosin (HE) staining showed vesicular

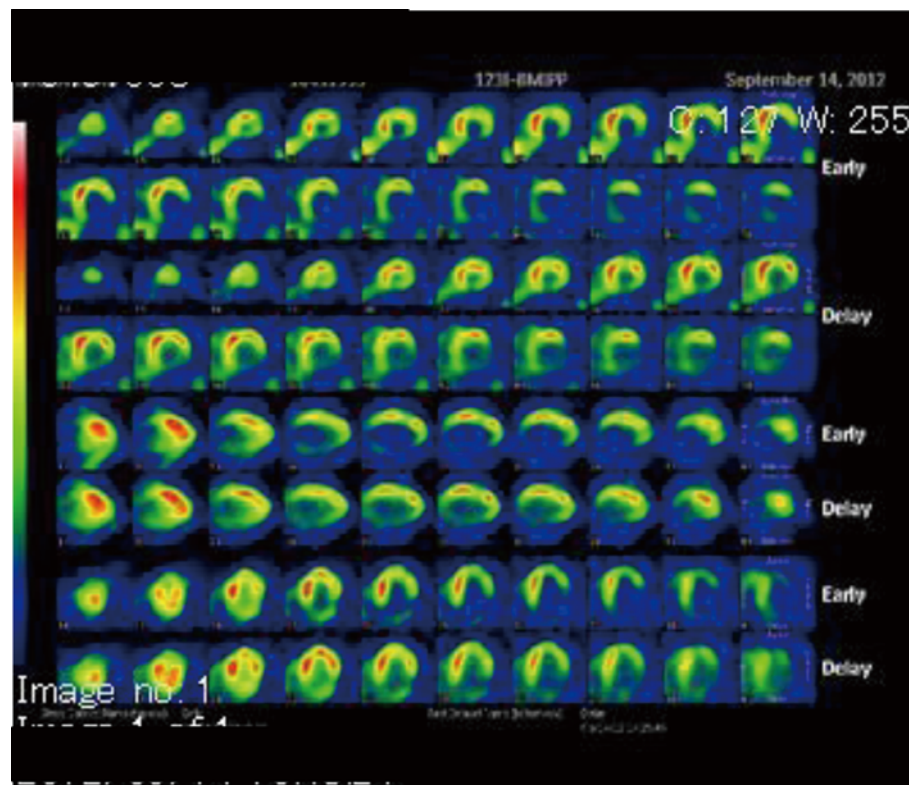


Fig. 6 SPECT images of  $^{123}\text{I}$  BMIPP in a patient with genetic ATGL deficiency.

vacuoles in cardiomyocytes. For the detection of lipid using oil red O staining, freshly frozen or formalin-fixed tissues can be used. It should be noted that paraffin treatments lose lipids themselves from the specimens. IMS technique can detect the spatial distribution of TG (Fig. 7) (13, 23). Table 2 summarized the laboratory tests and imaging modalities to detect myocardial TG deposition, even the number of genetic ATGL deficiency is ultra-rare and information is very limited. As shown in Fig. 2, abnormal intracellular metabolism of LCFAs is the major aspect for pathophysiology of TGCV, we think that BMIPP is useful for the diagnosis of this disorder. Nuclear imagings with not only BMIPP but also other tracers such as  $^{123}\text{I}$ -labeled meta-iodobenzylguanidine (MIBG) (24, 25) in patients with TGCV and other types of cardiomyopathy (26) are future and important research focuses. To detect myocardial TG deposition at the earlier stage of the disease, CT and MR spectroscopy seem feasible.

### Imaging modalities to detect coronary TG accumulation

#### 1. CT-based TG imaging

Fig. 8 shows the color-coded CT images (16) of short axial sections of the left anterior descending coronary artery in a TGCV patient with genetic ATGL deficiency, as performed for myocardium. For coronary arteries, color is coded by CT number (yellow: -25-0, orange: 0-40, green: 40-215, red: 215-700, white: 700-1200 HU) (M@XNET, Tokyo, Japan). When defined TG-containing tissue as < 40 HU, yellow or orange

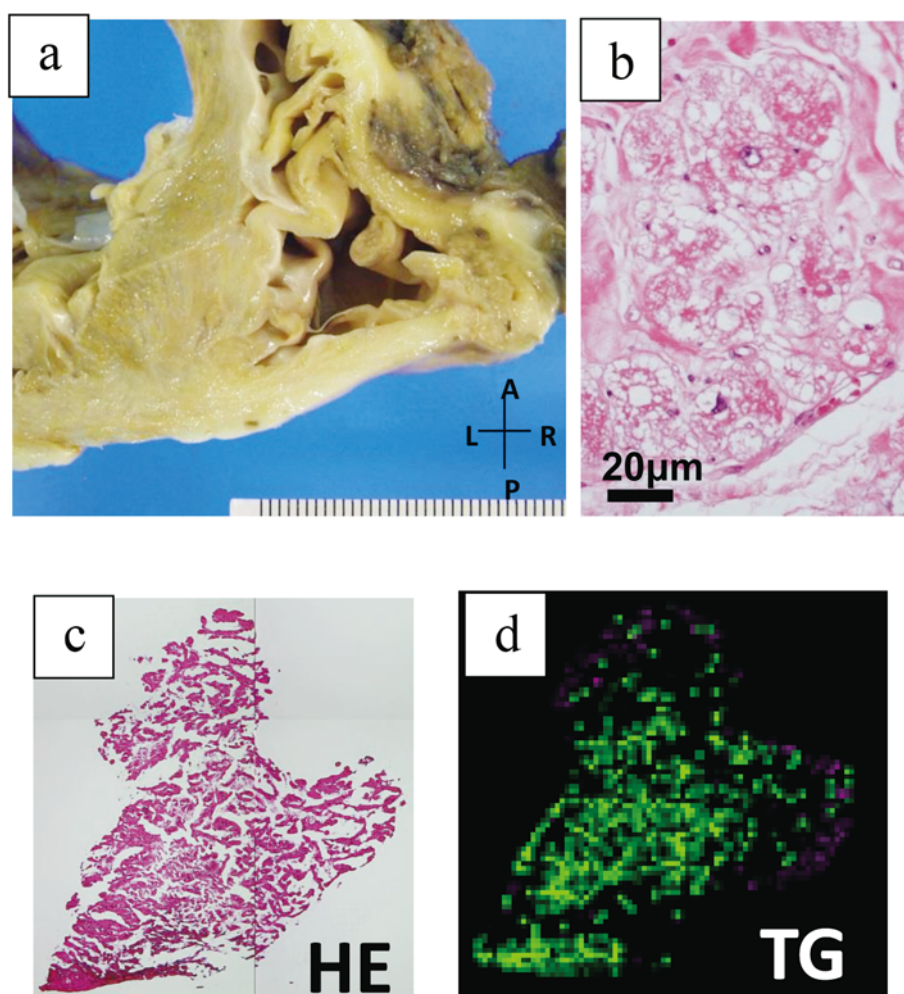
area indicates TG. Others roughly correlate as follows: white; calcification, red; luminal blood, green; coronary wall without calcification or TG. These images show abundant TG within the wall, distributing mainly in the adventitial side and protruding from outside toward inside in nodular, peninsular or bridging pattern in this patient. These findings are characteristic for TGCV patients. As comparison, color-coded images from normal coronary and coronary arteries with concentric stenosis in a non-TGCV patient are shown.

#### 2. Other modalities

Any other currently-available modalities including intravascular ultrasound (IVUS), optical coherence tomography (OCT), and others, do not focus on TG accumulated in SMCs, but cholesterol in macrophages.

### Future perspectives

We previously found the concept “TGCV” in ultra-rare cases with genetic ATGL deficiency. The following studies indicate that this concept can be applied to commonly-observed and intractable heart diseases. Because remedies targeting cholesterol have remarkably made progressed, it would be of significance to know how relevant TGCV is particularly in patients with residual risk for cardiovascular diseases. For this purpose, it needs to refine or develop imaging modalities focusing on TG in vascular SMCs to facilitate the diagnosis of TGCV.



**Fig. 7** a, b: Macroscopical (left) and microscopical (right) images of myocardium from a patient with genetic ATGL deficiency. Cardiomyocytes were filled with lipid droplets in cytoplasm in hematoxylin and eosin (HE) staining. c: HE staining of endomyocardial biopsy specimens from a patients homozygous for c.865C>T. d: IMS image of the same specimen as d. Green color denotes triglyceride. Panels a and b were published in our previous literature (5).

**Table 2** Summary for laboratory tests and imaging modalities for myocardium in TGCV

ECG	Appearance depending on cardiac morphology
UCG	Appearance depending on cardiac morphology
BMIPP scintigraphy	Specific for detection of abnormal LCFA metabolism
Cardiac CT	Useful for detecting TG accumulation even at the intial stage
Cardiac MRI	Useful for detecting adipose invasion and tissue damages, but less sensitive to cardiomyocyte steatosis compared with CT
MR spectroscopy	Useful for detecting TG accumulation, even at the intial stage, but some technical difficulties
Endomyocardial biopsy with imaging mass spectrometry	Specific for TG deposition, but invasive and not quantitative

The above description is based upon current technical levels of each modality.

#### Acknowledgments

This research was partially supported by research grants for rare and intractable diseases from the Japan Agency of Medical Research and Development (AMED) and from the Ministry of Health, Labor, and Welfare of Japan.

#### Sources of funding

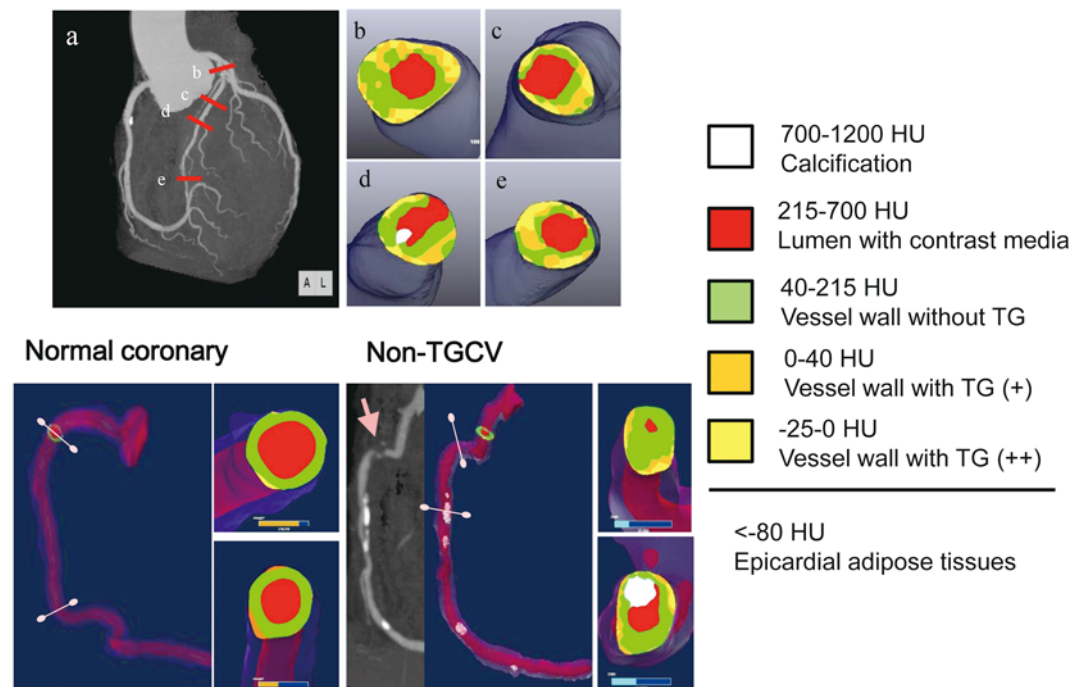
None.

#### Conflicts of interest

None.



## TGCV



**Fig. 8** CT-based TG imaging of short axial sections of the left anterior descending coronary artery in a TGCV patient with genetic ATGL deficiency (upper panels). Color is coded by CT number (yellow: -25-0, orange: 0-40, green: 40-215, red: 215-700, white: 700-1200 HU) (M@XNET, Tokyo, Japan). When defined TG-containing tissue as < 40 HU, yellow or orange area indicates TG accumulation. Others roughly correlate as follows: white; calcification, red; luminal blood, green; coronary wall without calcification or TG accumulation. These images (upper panels) show abundant TG within the wall, distributing mainly in the adventitial side and protruding from outside toward inside in nodular, peninsular or bridging pattern. Images from a patient with non-TGCV atherosclerosis and subject with normal coronary were shown as comparison in lower panels. It should be noted that epicardial and subcutaneous adipose tissues have CT numbers with -80 and -100 HU, respectively.

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