Triglyceride deposit cardiomyovasculopathy (TGCV) is a rare and intractable disease, first reported in Japanese patients with congestive heart failure (HF) requiring heart transplant. TGCV is characterized by the excessive accumulation of triglyceride (TG) in cardiomyocytes and vascular smooth muscle cells, which leads to coronary artery disease, HF, and arrhythmia. In TGCV, long-chain fatty acid (LCFA), a major energy source for the normal heart, accumulates as TG in cytoplasmic lipid droplets. In 2009, we launched the Japan TGCV study group to elucidate the pathophysiology of TGCV and have developed diagnostic procedures along with specific treatment. Single-photon emission computed tomography (SPECT) with iodine-123-β-methyl iodophenyl-pentadecanoic acid (BMIPP), a radioactive analogue for LCFA, is a useful diagnostic tool to detect impaired myocardial LCFA metabolism in TGCV. Since we posted the latest version of diagnostic criteria including the myocardial washout rate of BMIPP in SPECT in 2016, we have identified 138 patients with TGCV, 27 of whom have died. More recently, we developed a TGCV severity score consisting of specific questionnaires in order to assess symptoms and activities of daily living in patients with TGCV.

Keywords: Diagnostic criteria, Iodine-123-β-methyl iodophenyl-pentadecanoic acid, Severity score, TGCV questionnaire, Triglyceride deposit cardiomyovasculopathy, Washout rate
Triglyceride deposit cardiomyovasculopathy (TGCV) is a novel disease concept that was discovered in Japanese cardiac transplant recipients (1). Patients with TGCV have severe heart failure (HF), arrhythmia, and coronary artery disease (CAD) (1-3). Based on our clinical studies, the diagnostic criteria for TGCV have been reported by the Japan TGCV study group, where the increasing importance of iodine-123-β-methyl iodophenyl-pentadecanoic acid (BMIPP) single-photon emission computed tomography (SPECT) has been clarified. Here, we provide current information on TGCV and the activities of the Japan TGCV study group.

Definition, classification, and pathophysiology of TGCV

The phenotype of TGCV was primarily reported in patients with severe HF and genetic mutations of adipose triglyceride lipase (ATGL) (1), which is a rate-limiting enzyme for intracellular hydrolysis of TG and release of long-chain fatty acid (LCFA) as a major cardiac energy source (4). TGCV is characterized by excessive TG accumulation in both myocardium and coronary arteries, and the subtypes are classified into primary TGCV (P-TGCV, with ATGL mutations) and idiopathic TGCV (I-TGCV, without ATGL mutations) (5).

Genetic causes and etiologies of the latter are unknown. I-TGCV was initially identified in the autopsied study with diabetic patients (6) and we recently reported that ATGL activities in peripheral leukocytes were markedly reduced in some patients with I-TGCV (7). In TGCV, abnormal metabolism of intracellular TG and LCFA such as ATGL dysfunction leads to lipotoxicity and energy failure in affected cells and tissues, resulting in HF, CAD, and arrhythmia (3, 7).

Latest version of diagnostic criteria for TGCV and number of patients identified

In September 2016, we posted the latest version of diagnostic criteria for TGCV (8). As shown in Table 1, these criteria include 2 major items (2 points each) and 2 minor items (1 point each). Four points or more and 3 points indicate definite and probable TGCV, respectively. The major items refer to TG deposition in myocardium and coronary arteries, which is the pathological basis of TGCV. The two minor items are Jordans’ anomaly (9, 10) and diabetes mellitus. To date, 138 patients with TGCV have been identified from 7 institutes and hospitals in Japan. Unfortunately, 27 patients have died. These data indicate that TGCV is a rare and intractable disease.

| Items | Clinical findings |
|-------|------------------|
| 1. Major items (2 points) | 1.1 Myocardial TG deposition or impaired LCFA metabolism |
| | At least one of the following: |
| | 1) Myocardial TG deposition by biopsy specimens (a) |
| | 2) Myocardial TG deposition by MR spectroscopy |
| | 3) Decreased washout rate (<10%) of BMIPP |
| 1.2 Diffuse narrowing coronary arteries demonstrated by CAG, CT angiography (b) | |
| 2. Minor items (1 point) | 2.1 Jordans’ anomaly (apparent vacuoles of about 1 micrometer in size) of polymorphonuclear leucocytes in peripheral blood smear (c) |
| | 2.2 Diabetes mellitus (d) |
| Diagnosis of TGCV | (1) 4 points or more | Definite TGCV |
| | Primary TGCV, if with ATGL mutation |
| | Idiopathic TGCV, if without ATGL mutation (e) |
| (2) 3 points | Probable TGCV |
| | If ATGL mutations confirmed, definite TGCV considered |

(a) For tissue TG contents examination, frozen sections with osmium fixation, but not paraffin sections, should be used for prevention of lipid elution
(b) The presence or absence of a significant stenosis is not considered
(c) For difficult cases, May-Giemsa staining slides of peripheral blood smear will be evaluated by the Japan TGCV study group
(d) According to the diagnostic criteria of DM by the Japan Diabetes Society
(e) If no opportunity for genetic analyses for ATGL (i.e., deceased cases), the Japan TGCV study group can judge under clinicopathological datasets
Please feel free to contact our study group (E-mail: info@tgcv.org)

* Abbreviations: ATGL: adipose triglyceride lipase, BMIPP: iodine-123-β-methyl iodophenyl-pentadecanoic acid, CAG: coronary angiography, CT: computed tomography, LCFA: long chain fatty acid, MR: magnetic resonance, TG: triglyceride, TGCV: Triglyceride deposit cardiomyovasculopathy

(Note: English version of table has been provided by the authors)
cardiovascular disease.

Significance of washout rate of BMIPP-SPECT in the diagnosis of TGCV

BMIPP-SPECT is used to evaluate metabolism of LCFA in cardiomyocytes (11). LCFA is an essential energy source of the normal heart. After LCFA is taken up by cardiomyocytes, it is either synthesized to TG or being undergone beta oxidation to be energy-utilized, and BMIPP takes similar intracellular dynamics (11, 12). However, in patients with TGCV, once LCFA is pooled as TG, it remains inside the cell without being hydrolyzed (3). Hence, patients with TGCV have markedly decreased washout rate (WOR) of BMIPP (13), which is unrelated to local myocardial uptake abnormality (Fig. 1). In order to calculate the WOR of BMIPP, acquisition of delayed imaging up to 240 minutes after intravenous administration of tracer is performed in addition to early imaging at less than 30 minutes. After constructing polar map displays from short-axis, early and delayed SPECT imaging, WOR is calculated from the mean tracer counts (13). BMIPP-SPECT for diagnosis of TGCV should be avoided in the acute phase of acute coronary syndrome, such as acute myocardial infarction and unstable angina pectoris, because both washout and fill-in of BMIPP in delayed imaging have been reported in the acute phase of myocardial ischemia (14-16), thereby impeding accurate evaluation of TGCV.

Fig. 1 123I-BMIPP SPECT images in a TGCV patient.
A: Short-axis, vertical long-axis, and horizontal long-axis tomographic images.
B: Displays on a circumferential polar plot.
Detection of myocardial small lipid droplets
In some cases with TGCV, lipid droplets in cardiomyocytes are too small to be detected by lipid staining such as Oil red O. Hara et al. reported that the overexpression of perilipin (PLIN) 2, a lipid droplet maintenance protein, was a possible biomarker candidate in the context of lipid metabolism using fibroblasts from TGCV patients (17). Therefore, we can detect small lipid droplets as a PLIN 2 protein mass in cardiomyocytes (Fig. 2B), indicating that PLIN2 may be a useful histological protein marker for cardiomyocyte steatosis.

Differential diagnosis of TGCV
It is important to differentiate TGCV from other cardiovascular diseases as follows: dilated cardiomyopathy, hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, mitochondrial cardiomyopathy, alcoholic heart disease, metabolic myocardial disorders (e.g., Fabry disease, Pompe disease, and cholesteryl ester storage disease). Furthermore, because a mild reduction of BMIPP WOR was reported in chronic hemodialysis patients (18), it would be of interest to know whether TGCV phenotype might exist among patients with chronic kidney disease, as reported in our postmortem study (6).

Development of severity score for TGCV
In order to assess symptoms of this newly identified disease, we collected clinical symptoms through interviews with TGCV outpatients in the Osaka University Hospital. Based upon the information, we developed 2 sets of TGCV-specific questionnaires: One set is for symptoms (the upper panel in Table 2) and the other for activities of daily living (ADL) (the lower panel in Table 2).

The former includes 4 sections as follows: 1) ischemic heart symptoms, 2) arrhythmia symptoms, 3) heart failure or energy failure symptoms, and 4) peripheral symptoms. Each section includes 3-5 questions (Qs). The point for each answer ranges from 0 (Never) to 4 (Always) (please see the footnote of the upper panel). As shown in Table 2 with input example, if patient’s answers are “Frequently”, “Frequently”, “Frequently”, “Always” and “Frequently” for Qs1-5 in the first section, the points would be 3, 3, 3, 4 and 3, respectively. The subscore of this first section is total points of the five answers (16 points). Other subscores are summed as the same as the first section. The severity score for symptoms is the total of subscores from four sections. Thus, TGCV severity score for symptoms would be 37 points in this input example.

The latter includes 6 sections as follows: 1) diet and cooking, 2) physical cleanliness, 3) excretion, 4) daily life movement/change of clothes, 5) light physical work, and 6) walking. Each answer is rated on a 4-point scale (0-On my own, 1-Slightly hard, 2-Very hard, 3-impossible) (please see the footnote of lower panel). As shown in the lower panel of Table 2, the severity score for ADL is summed as that for symptoms.

The significance of patient-reported outcomes has been acknowledged for the assessment of clinical trials and development of therapy (19, 20). Although it is necessary to examine the reliability and validity of the questionnaires and
### Table 2  Severity Scores for Triglyceride Deposit Cardiomyovasculopathy (TGV)

#### 1) Questionnaire for symptoms

| Clinical classification | No. Questions for Symptoms | Point | Subscore | Severity score |
|-------------------------|---------------------------|-------|----------|----------------|
| **Section 1** Ischemic heart symptom | Q1: Do you have chest pain (angina pain) and heaviness and squeezing in the chest? | 3 | | |
| | Q2: Have you taken sublingual nitroglycerin? | 3 | | |
| | Q3: Have you had the following symptoms before you have chest pain? (feeling like choking; feeling that something is welling up in the throat; feeling that your back is getting hot, etc.) | 3 | 16 | |
| | Q4: Have you experienced symptoms of the chest, dyspnea, or shortness of breath, regardless of whether there is exertion or you are at rest? | 4 | | |
| | Q5: Have you had pain which spreads to the back, shoulders, or jaw? | 3 | | |
| **Section 2** Arrhythmia symptom | Q6: Have you experienced palpitations such as a feeling of pounding or fluttering to your heartbeat? | 3 | | |
| | Q7: Have you experienced that your pulse is suddenly racing? | 0 | | |
| | Q8: Have you ever had a feeling of weakness in the hands or feet (i.e., feel heavy in the arms or legs)? | 3 | | |
| **Section 3** Heart failure, energy failure symptom | Q9: Do you feel easily fatigued, general malaise, etc.? | 4 | | |
| | Q10: Have you experienced edema on your face, hands, or feet? | 4 | | |
| | Q11: Have you felt that your breathing is better sitting up rather than lying down at night? | 0 | | |
| | Q12: Have you ever felt weak in the hands or feet (i.e., feel heavy in the arms or legs)? | 3 | | |
| | Q13: Do you always feel that your temperature is low? | 1 | | |
| **Section 4** Peripheral symptom | Q14: Have you ever felt that your fingers or toes get too cool (cold sensation in the extremities)? | 1 | | |
| | Q15: Have you ever had numbness in the fingers or toes or your entire hands or feet? | 0 | | |
| | Q16: Have you ever felt dullness in the fingers or toes or your entire hands and feet? | 0 | | |
| | Q17: Have you ever had a cramp in your leg or toe? | 3 | | |
| | Q18: For respondents receiving diabetes treatment - have you ever experienced a hypoglycemic episode? | 2 | | |

Point definition for patients’ answers: O, never; 1, rarely; 2, sometimes; 3, frequently; 4, always.

#### 2) Questionnaire for Activities of Daily Living (ADL)

| Classification | No. Questions for ADL | Point | Subscore | Severity score |
|----------------|----------------------|-------|----------|----------------|
| **Section 1** Diet, cooking | Q1: Can you prepare your meals including cooking? | 1 | | |
| | Q2: Can you have a meal by yourself? | 0 | | |
| | Q3: Can you open a can or open the lid of a plastic bottle by yourself? | 1 | | |
| | Q4: Can you straighten up and wash the dishes and clear the table after a meal? | 2 | | |
| **Section 2** Physical cleanliness | Q5: Can you take a bath or shower by yourself? | 2 | | |
| | Q6: Can you reach to wipe your whole body by yourself? | 0 | | |
| | Q7: Can you do facial cleansing or toothbrushing by yourself? | 0 | | |
| | Q8: Can you do your hair (using a tool including comb and hairdryer) by yourself? | 0 | | |
| **Section 3** Excretion | Q9: Can you go to the bathroom without difficulty by yourself in the daytime? | 0 | | |
| | Q10: Can you go to the bathroom by yourself at night? | 0 | | |
| | Q11: Can you get up from a toilet seat by yourself? | 0 | | |
| | Q12: Can you get up from a toilet seat by yourself in a bathroom? | 0 | | |
| **Section 4** Daily lifemovement change of clothes | Q13: Can you hang out the laundry and take in it? | 0 | | |
| | Q14: Can you change your clothes by yourself? | 1 | | |
| | Q15: Can you put on your shoes (footwear) by yourself? | 2 | | |
| | Q16: Can you clean your room? | 2 | | |
| **Section 5** Light laborious work | Q17: Can you walk in your house without restrictions? | 0 | | |
| | Q18: Can you carry heavy shopping bag, baggage and bag in your hand or on your shoulder? | 3 | | |
| | Q19: Can you go shopping or walking alone? | 1 | | |
| | Q20: Can you perform light exercise in a room (e.g., gymnastics done to commands and music on the radio [television])? | 2 | | |
| **Section 6** Walk | Q21: Can you walk around 100 to 200 m on flat land? | 1 | | |
| | Q22: Can you walk rapidly (in step with a healthy person)? | 1 | | |
| | Q23: Can you walk on a slope without difficulty? | 2 | | |
| | Q24: Can you go up and down the stairs to the second floor? | 2 | | |

Point definition for patients’ answers: O, on my own; 1, slightly hard; 2, very hard; 3, impossible.

(Note: English version of table has been provided by the authors)
to compare the results with those of other subjective instruments including the Minnesota Living with Heart Failure questionnaire (21) and the 36-item Short Form (22), we believe that the developed specific severity score will be useful in clinical trials and studies of patients with TGCV.

Issues to be resolved

Following points are important focus for future researches:

1. Etiologies and genetic causes of I-TGCV
Our previous report (23) indicated that heterozygous carriers of ATGL mutation did not show apparent cardiovascular or neurological symptoms, compared with non-carriers. The mechanism underlying down-regulation of ATGL activities of I-TGCV and possible involvement of other lipases and related enzymes is of significance to elucidate.

2. As mentioned recently (10), vacuolar formation was observed in less than 10% of leukocytes in I-TGCV, in contrast to P-TGCV with almost all leucocytes manifesting Jordans’ anomaly (24). Any screening methods to detect Jordans’ anomaly and measure ATGL protein in I-TGCV need to be developed.

3. Continued validation of diagnostic criteria for TGCV
The number of TGCV patients is still limited. During the course of elucidation of all issues above, diagnostic criteria should be kept updated.

Finally, the Japan TGCV study group recently reported that medium-chain fatty acids had a therapeutic effect in a mouse model for TGCV (25) and have already finished phase I and I/IIa clinical trials (NCT 02502578, NCT 02830763). A next-phase clinical trial is now in preparation.

Author contribution

HM, CH, YI, ML, KK, and KH wrote the manuscript. HM, YI, MaH, TI, and HN are members of the task force on diagnostic criteria. CH, HM, and ML are members of the task force on severity score. YN, JK, ES, YaN, KS, SK, KK, TI, TA, and KK provided patient information and contributed to the discussion. KH is the principal investigator for the Japan TGCV study group.

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Conflicts of interest

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