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LETTER TO THE EDITOR

# Distinct myocardial triglyceride lipolysis pathways in primary and idiopathic triglyceride deposit cardiomyovasculopathy

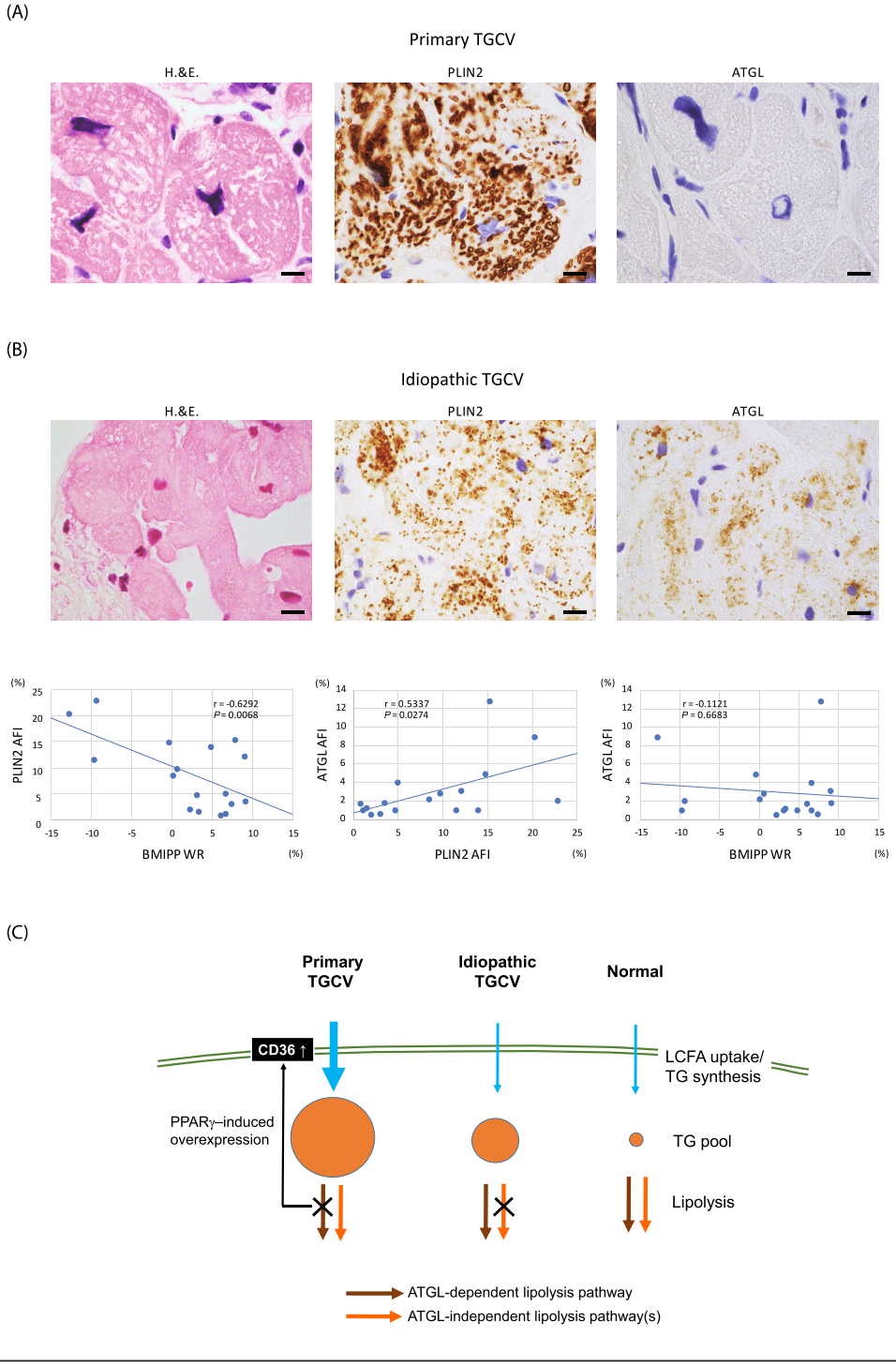
Triglyceride (TG) is a major energy source for a normal heart. Myocardial lipolysis is a crucial step to hydrolyse TG and release long-chain fatty acid (LCFA) for mitochondrial β-oxidation to produce ATP. Therefore, it is of significance to elucidate its regulatory pathway(s) in order to understand pathophysiology of heart failure (HF). Triglyceride deposit cardiomyovasculopathy (TGCV) is a rare, emerging cardiovascular disorder first reported in patients with HF with massive cardiomyocyte steatosis who required cardiac transplantation.[1] In TGCV, defective intracellular TG lipolysis results in TG deposition and energy failure, mainly in cardiomyocytes and coronary smooth muscle cells.[2,3] TGCV is classified into primary and idiopathic types with and without genetic mutation of *PNPLA2* encoding adipose triglyceride lipase (ATGL), respectively.[3] In both types, defective TG lipolysis can be evaluated using scintigraphy by calculating the washout rates (WR) of $^{123}$I-β-methyl-p-iodophenyl-pentadecanoic acid (BMIPP),[2,3] an established radiopharmaceutical of LCFA analogue.[4,5] After intravenous administration, BMIPP is taken up by cell surface transporters including CD36. Approximately 90% of cellular BMIPP form TG-BMIPP and are incorporated into TG pool. Then, TG-BMIPP is hydrolysed by intracellular lipases including ATGL, and eventually, its catabolites are washed out from cardiomyocytes.[2–4] Thus, BMIPP-WR, defined as a % count reduction between early and delayed images in myocardial scintigraphy,[5] reflects lipolysis of TG in the heart.[2,3]

We developed the diagnostic criteria for TGCV in which low BMIPP-WR was included as an essential item and have identified ~200 patients by December 2021. Patients with TGCV exhibit adult-onset HF, diffuse coronary artery disease (CAD), and ventricular arrhythmia with high mortality in our recent registry study.[6] Although they received standard medical and interventional therapies for HF and CAD, the 5-year-overall and cardiovascular event-free survival rates of patients with TGCV were approximately 70% and 53%, respectively.[6] The major cause of death was cardiovascular events. Non-fatal cardiovascular events included revascularization, stroke, and hospitalization of HF or device implantation.[6] For a possible specific treatment, a phase IIb/III clinical trial with a first-in-class orphan drug, of which active ingredient is tricaprin/trisdecanoin, is underway (jRCT2051210177), after proving that tricaprin/trisdecanoin facilitated myocardial lipolysis in patients with TGCV.[7,8]

In primary TGCV (P-TGCV), inherited in the autosomal recessive fashion, a homozygous genetic defect of ATGL, an essential molecule to hydrolyse intracellular TG and release LCFA for mitochondrial β-oxidation, causes cellular steatosis.[1–3] However, the mechanism(s) underlying defective intracellular lipolysis and subsequent TG deposition remains unknown in idiopathic TGCV (I-TGCV). Here, we report pathological characteristics of endomyocardial biopsy (EMB) specimens from TGCV patients and discuss the regulation of myocardial TG deposition in I-TGCVs compared with that in P-TGCVs.

A series of 18 consecutive EMB specimens from patients with TGCV (1 primary and 17 idiopathic types) referred from 7 hospitals to the Department of Pathology, National Cerebral and Cardiovascular Center (NCVC), the core laboratory of myocardial pathology for the Japan TGCV study group, between August 2020 and December 2021 were analysed. BMIPP scintigraphy and measurement of BMIPP-WR were performed according to the recommendation by the Japan Society of Nuclear Cardiology.[5] The P-TGCV patient was a 34-year-old man with HF, homozygous for a large deletion of *PNPLA2* (Gene ID: LC508023). The patient's BMIPP-WR was −4.5% (<10% BMIPP-WR, the cutoff for TGCV diagnosis[5]). The 17 I-TGCV patients included 15 men and 2 women (mean age: 66.1 ± 12.7 years). They all presented with HF or diffuse CAD, and the mean BMIPP-WR was 2.0 ± 6.7%. Following routine analyses with haematoxylin and eosin (H&E) staining, paraffin-embedded sections of EMB specimens were immunostained, and the area fractions of immunoreactivities (AFIs) were evaluated using two

**Figure 1** (A) Microscopic features of EMB specimens in primary TGCV (scale bar: 20 μm). (B) Representative microscopic features of EMB specimens in idiopathic TGCV (scale bar: 20 μm) and a graph showing the correlation between the washout rate of BMIPP in myocardial scintigraphy, the area fraction of immunoreactivities (AFI) of PLIN2, and ATGL. Each dot denotes the mean of AFIs calculated from 3 different high power fields of view. Anti-PLIN2 (Cat. No. 690102; 1:100, PROGEN, Germany) and anti-ATGL (Cat. No. 2138; 1:100. Cell Signalling, Danvers, MA, USA) antibodies were used for immunostaining. (C) In primary TGCV, massive TG accumulation is caused by the defect in ATGL-dependent lipolysis and increased uptake of LCFA through PPARγ-induced overexpression of CD36, a cell surface LCFA transporter. In idiopathic TGCV, despite possible upregulation of ATGL by metabolic adaptation to prevent excess TG deposition, increased TG pool and very low washout rate of BMIPP are observed. ATGL-dependent and putative ATGL-independent lipolysis pathways might be involved in TG deposition in TGCV. ATGL, adipose triglyceride lipase; BMIPP, $^{123}$I-β-methyl-*p*-iodophenyl-pentadecanoic acid; EMB, endomyocardial biopsy; LCFA, long-chain fatty acid; PLIN2, perilipin-2; PPARγ, peroxisome proliferated activated receptor-γ, TG, triglyceride; TGCV, triglyceride deposit cardiomyovasculopathy.





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antibodies: ATGL responsible for P-TGCV and perilipin-2 (PLIN2), a protein coating lipid droplets in non-adipocytes to detect intracellular lipid deposition in cardiomyocytes, alternative to lipid staining owing to limited availability of frozen EMB samples. Correlations between PLIN2- or ATGL-AFI and BMIPP-WR were evaluated using Pearson's correlation analysis in I-TGCV. *P*-values $<0.05$ were considered statistically significant, and statistical analysis was performed using JMP version 14.3.0 (SAS Institute Inc., Tokyo, Japan). This study was approved by the Ethics Committee of NCVC.

EMB specimens from the P-TGCV patient exhibited severely hypertrophied cardiomyocytes full of multiple large lipid droplets negative for ATGL (*Figure* 1A). The PLIN2-AFI was 54.2 ± 2.9% (mean ± SD from three different high power fields). Cardiomyocytes in the I-TGCV group were smaller than those from P-TGCV, but possessed numerous numbers of lipid droplets positive for both PLIN2 and ATGL (*Figure* 1B). Lipid droplets in I-TGCV were small and sometimes rarely observed using H&E staining. Correlation analyses in I-TGCV patients demonstrated negative correlations between BMIPP-WR and PLIN2-AFI ($r = -0.6292$, $P = 0.0068$). ATGL-AFI positively correlated with PLIN2-AFI ($r = 0.5337$, $P = 0.0274$). No correlation existed between BMIPP-WR and ATGL-AFI, indicating that defective myocardial lipolysis is likely independent of ATGL expression in I-TGCV.

Myocardial lipid deposition is determined by substrate uptake, TG synthesis, and lipolysis (*Figure* 1C). In P-TGCV, as reported previously,[9] massive TG accumulation was caused by the defect in ATGL-dependent lipolysis and increased LCFA uptake through peroxisome proliferated activated receptor-γ-induced CD36 overexpression. In I-TGCV specimens investigated, the ATGL expression was conserved and positively correlated with the degree of lipid deposition (PLIN2-AFI). Even though ATGL was overexpressed possibly by metabolic adaptation to prevent excess TG deposition, intracellular TG deposition and very low BMIPP-WR were observed. These results indicated that a putative ATGL-independent lipolysis pathway may be involved in regulating TG deposition in I-TGCV. The existence of such an ATGL-independent lipolysis pathway is also supported by the evidence that tricaprin/trisdecanoin improved myocardial BMIPP-WR and reduced TG deposition in ATGL-knockout mice.[10] Further studies are required to identify the molecule(s) involved in ATGL-independent lipolysis, particularly causing I-TGCV.

The present study indicates distinct myocardial TG lipolysis pathways in primary and idiopathic TGCV, which is a rare but important cause of HF. Our findings provide information to understand heterogeneity of myocardial TG lipolysis pathways and complexed regulatory mechanisms for lipid droplet formation in HF.

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## Conflict of interests

KH has held the position of Joint Research Chair in collaboration with Toa Eiyo Ltd. (Tokyo, Japan) since February 2021 and has served as a medical advisor for Toa Eiyo Ltd. since December 2021. KH has a pending patent. The other authors have no competing interests.

Yoshihiko Ikeda
*Department of Pathology, National Cerebral and Cardiovascular Center, Osaka, Japan*
*Department of Triglyceride Science, Graduate School of Medicine, Osaka University, Osaka, Japan*

Mikio Shiba
*Cardiovascular Division, Osaka Police Hospital, Osaka, Japan*

Hiroyuki Yamamoto
*Department of Cardiovascular Medicine, Narita-Tomisato Tokushukai Hospital, Chiba, Japan*

Tomonori Itoh
*Department of Internal Medicine, Division of Cardiology, Iwate Medical University, Iwate, Japan*

Takahiro Okumura
*Department of Cardiology, Graduate School of Medicine, Nagoya University, Nagoya, Japan*

Yusuke Nakano
*Department of Cardiology, Aichi Medical University, Nagakute, Japan*


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Hideyuki Miyauchi
*Department of Cardiovascular Medicine, Chiba University Graduate School of Medicine, Chiba, Japan*

Tomomi Ide
*Department of Cardiovascular Medicine, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan*

Sohsuke Yamada
*Department of Pathology and Laboratory Medicine, Kanazawa Medical University, Uchinada, Japan*

Hiroyuki Hao
*Department of Pathology and Microbiology, Division of Human Pathology, Nihon University School of Medicine, Tokyo, Japan*

Seiya Kato
*Division of Pathology, Saiseikai Fukuoka General Hospital, Fukuoka, Japan*

Ken-ichi Hirano
*Department of Triglyceride Science, Graduate School of Medicine, Osaka University, Osaka, Japan*
*E-mail: khirano@cnt-osaka.com*

On behalf of the Japan TGCV study group

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