Triglyceride Deposit Cardiomyovasculopathy

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Keywords
Adipose triglyceride lipase · Heart failure · Long-chain fatty acids · Triglyceride deposit cardiomyovasculopathy · Triglyceride

Abbreviations

ATGL	Adipose triglyceride lipase
BMI	Body mass index
BMIPP	β-Methyl-p-[123I]-iodophenyl-pentadecanoic acid
CABG	Coronary artery bypass grafting
CTx	Cardiac transplantation
HF	Heart failure
LCFAs	Long-chain fatty acids
LV	Left ventricle
NLSD-I	Neutral lipid storage disease with ichthyosis
NLSD-M	Neutral lipid storage disease with myopathy
PCI	Percutaneous coronary intervention
SMCs	Smooth muscle cells
SPECT	Single-photon emission computed tomography
TG	Triglyceride
TGCV	Triglyceride deposit cardiomyovasculopathy
WOR	Washout rate

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11.1 Case Reports

11.1.1 Case 1: Primary Triglyceride Deposit Cardiomyovasculopathy (TGCV) with Adipose Triglyceride Lipase (ATGL) Mutation

A 41-year-old man was admitted due to palpitation and ventricular tachycardia. His body measurements were as follows: height, 181 cm; body weight, 58.6 kg; and body mass index (BMI), 18 kg/m^2. Blood pressure was 106/70 mmHg. Physical examination showed no skin lesions but showed mild muscle weakness of his right arm. A peripheral blood smear showed vacuoles in polymorphonuclear leukocytes called Jordans' anomaly (Fig. 11.1A). Plasma triglyceride (TG) level

T. Oohashi et al. (eds.), *Human Pathobiochemistry*, https://doi.org/10.1007/978-981-13-2977-7_11

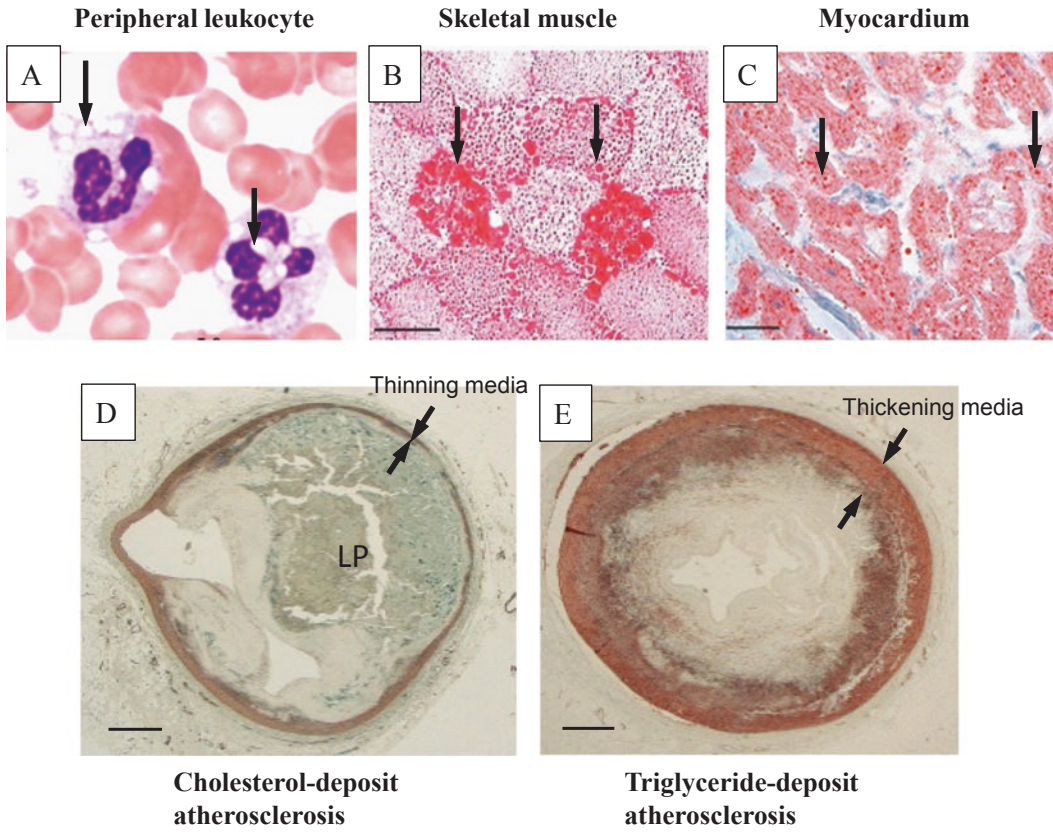

Fig. 11.1 Lipid deposition in peripheral leukocytes, skeletal muscle, myocardium, and coronary arteries in a patient (Case 1) with primary TGCV. Panel A shows peripheral blood smear with May-Giemsa staining. Arrows indicate vacuolar formation in neutrophils. Panel B shows oil red O staining of a biopsy specimen obtained from the patient's atrophied arm, with lipid droplets of predominantly type I fibers (arrows). All red dots denote lipid droplets. The scale bar represents 40 μm. Panel C shows oil red O-positive numerous vacuoles in the cytoplasm of cardiomyocytes (arrows) in the myocardium of the explanted heart after cardiac transplantation. The scale bar represents 50 μm. Panel D: cholesterol-deposit atherosclerosis had the following characteristics: focal and eccentric stenosis with large lipid pool (LP in the figure) paucity of SMCs in the fibrous cap and shoulders of the lesion and disrupted internal elastic lamina and thin medial layer (arrow) resulting in remodeling. In contrast, the patient with TG-deposit atherosclerosis showed diffuse concentric stenosis of the coronary arteries. The majority of foam cells were SMCs in thick intima and media (arrow in panel E)

was 137 mg/dl (normal value 30–150 mg/dl), and total cholesterol 160 mg/dl (normal value 51–219 mg/dl) was normal. Muscle biopsy revealed vacuolation positive for oil red O staining predominantly in type I fibers (Fig. 11.1B). Chest X-ray and echocardiography showed cardiomegaly with dilatation and reduced ejection fraction of the left ventricle (LV). Endomyocardial biopsy specimens showed massive vesicular vacuoles positive for oil red O in cardiomyocytes. The mitochondrial structure was normal. Myocardial scintigraphy with β-methyl-p-[123I]-iodophenyl-pentadecanoic acid (BMIPP) showed marked reduction of washout rate (WOR), suggesting abnormal metabolism of long-chain fatty acids (LCFAs), which are a major energy source for the normal heart.

Congestive heart failure (HF) developed progressively and became catecholamine-dependent within a couple of years. A left ventricular assist device was implanted, and the patient was registered as a heart transplant candidate on a waiting list. His passaged skin fibroblasts showed massive accumulation of TG and were used as a

model for investigating abnormalities in cellular metabolism and elucidating the underlying mechanism. Eventually, the patient received cardiac transplantation (CTx). Because the biochemical analysis of explanted heart showed marked accumulation of TG in both myocardium and coronary arteries, we named this novel phenotype as TGCV (Hirano et al. 2008; Hirano 2009).

Genetic tests revealed that this patient was homozygous for the loss-of-function mutation in the *PNPLA2* gene encoding ATGL, which is the rate-limiting enzyme of intracellular hydrolysis of TG. The pathological analysis of explanted heart showed vacuoles observed in cardiomyocytes and stained positive with oil red O (Fig. 11.1C). Vacuoles positive for oil red O were also observed in the cytoplasm of endothelial cells and smooth muscle cells (SMCs) of the media of the coronary arteries. In contrast to usual cholesterol-deposit atherosclerosis with focal and eccentric stenosis (Fig. 11.1D), his coronary arteries with TG deposition showed diffuse and concentric stenosis (Fig. 11.1E) (Hirano 2009; Ikeda et al. 2014a, b).

11.1.2 Case 2: Idiopathic TGCV Without ATGL Mutation

A 69-year-old male was admitted to our hospital due to acute chest pain. His body measurements were as follows: height, 157 cm; body weight, 47.1 kg; and BMI, 19 kg/m^2. Blood pressure was 108/46 mm Hg. His medical history included diabetes mellitus, hypertension, and hyperlipidemia. His lipid profiles were as follows: TG level 57 mg/dl (normal value 30–150 mg/dl), high-density lipoprotein cholesterol 53 mg/dl (normal value 40–80 mg/dl), and low-density lipoprotein cholesterol 54 mg/dl (normal value <140 mg/dl). Coronary angiograms showed diffuse and narrowing arteries (Fig. 11.2A). He was diagnosed as having unstable angina pectoris and subsequently underwent percutaneous coronary intervention (PCI). Despite several rounds of PCI and coronary bypass surgery (CABG), his chest pain was intractable. A frontal chest X-ray showed enlargement of cardiothoracic ratio. Electrocardiogram showed a normal sinus rhythm (80 beats/min), complete right bundle branch block, and multisource premature ventricular contractions. Echocardiography showed akinesis of the basal-mid lateral-posterior segment of the left ventricular wall and the severe apical hypokinesis with enlarged cavity and reduced ejection fraction of LV. Myocardial scintigraphy with BMIPP showed a marked reduction of WOR (Fig. 11.2B), as similar to those observed in patients with primary TGCV.

Genetic test showed no mutations in the exons and introns of genes encoding ATGL or its coactivator CGI-58; however the ATGL activity was very low in his peripheral leukocytes (Takagi et al. 2018). Eventually, the patient died suddenly.

11.2 Diagnosis

11.2.1 Definition and Classification

TGCV is a novel disease concept that we found in Japanese CTx candidates in 2008. Probands carried mutations in the *PNPLA2* gene encoding ATGL. ATGL is the major enzyme that catalyzes the initial rate-limiting step of intracellular TG hydrolysis to release free nonesterified long-chain fatty acids (LCFAs), which are an essential energy source for the normal heart. Patients with TGCV show ectopic accumulation of triglycerides in cardiomyocytes and SMCs resulting from abnormal intracellular metabolism of triglycerides and LCFA. In TGCV, cardiomyovascular TG deposition is not related to BMI or body weight, which reflects the TG accumulation in adipose tissues in the body. Cases 1 and 2 were not obese, which was consistent with the diagnosis of TGCV.

TGCV is classified into primary and idiopathic TGCV with and without genetic ATGL deficiency, respectively. Both types of TGCV patients suffer from severe HF, arrhythmia, and coronary artery disease caused by lipotoxicity and energy failure at cellular levels (Hirano 2009).

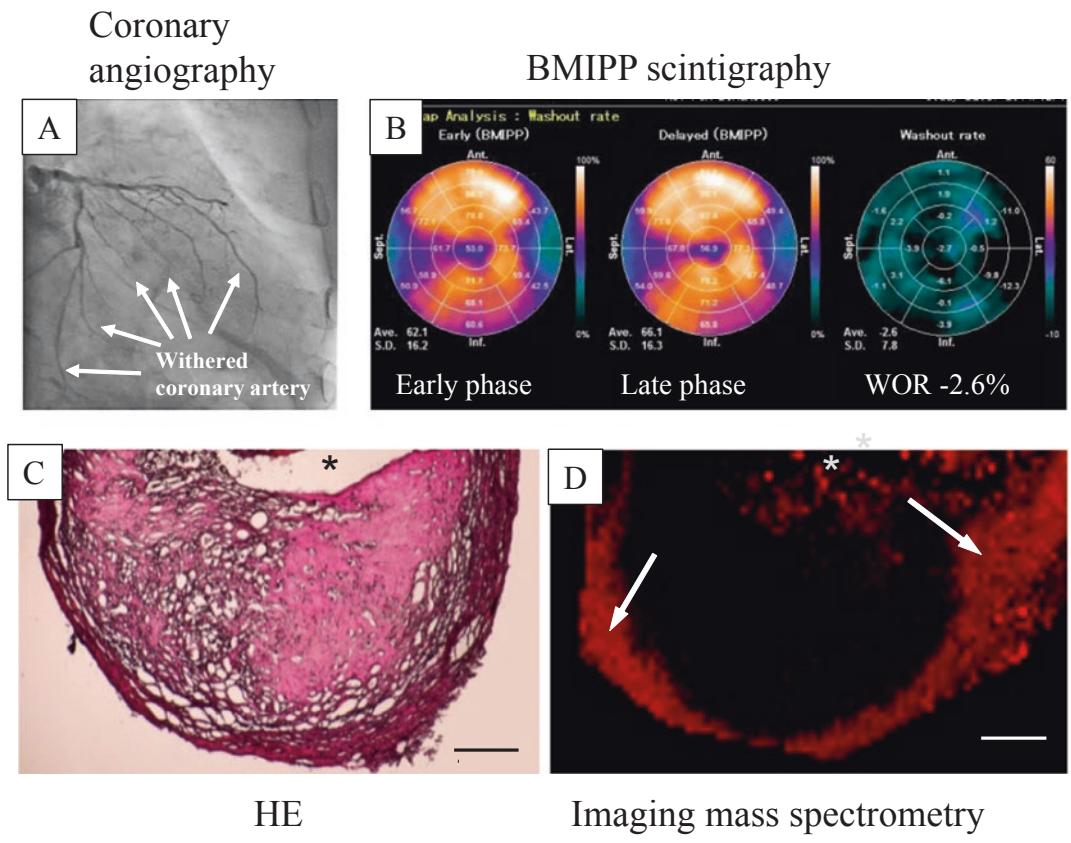

Fig. 11.2 Clinical imaging and pathological findings of an idiopathic TGCV patient (Case 2) without ATGL mutation. (**A**) Angiogram of left coronary artery shows diffuse narrowing like withered branches (arrow). (**B**) Bull's eye images of nuclear myocardial scintigraphy with BMIPP, a radioactive tracer for long-chain fatty acid (LCFA). Washout rate (WOR) was calculated as [BMIPP uptake (early phase)-remaining BMIPP (delay phase)/BMIPP uptake (early phase)] to evaluate the myocardial metabolism of LCFA. Orange colors in early and delayed phases denote the uptake of BMIPP. Blackout in the WOR image shows defective WOR (−2.4%, reference values >20%) (**C, D**). Spatial distribution of TG in coronary atherosclerotic lesions. Hematoxylin-eosin staining (**C**) and imaging mass spectrometry (**D**). Red dots (arrows) in **D** denote TG deposition, located in the fibrous cap and media of atherosclerotic lesions. Asterisks represent vascular lumen. The scale bars represent 500 μm

11.2.2 Clinical Signs and Symptoms

TGCV patients are healthy at birth but present with cardiac symptoms from their 20s to middle age. The symptoms of HF include palpitations, shortness of breath, dyspnea on exertion, fatigue, swelling (edema), and weight gain. As the disease progresses, more symptoms appear including difficulty breathing (dyspnea) at rest or when lying flat (orthopnea). Chest pain that radiates through the upper body occurs at rest and also at night, and does not respond to nitroglycerin or nitrites. Arrhythmia causes slow, irregular heartbeats or fainting (syncope). Many of patients have a history of cardiopulmonary arrest, and some have died of sudden cardiac death. In cases of primary TGCV, symptoms associated muscle weakness, and skeletal myopathy was frequently observed.

11.2.3 Laboratory and Imaging Data

In both primary and idiopathic TGCV, myocardial WOR of BMIPP calculated by early and delayed single-photon emission computed

tomography (SPECT) images is markedly reduced, indicating the impaired metabolism of LCFA and TG. Early BMIPP-SPECT images can be used to detect the severity of myocardial damage and coronary artery lesions (Hirano et al. 2015). Coronary CT angiograms with TG imaging show diffuse narrowing stenosis with outside-in TG deposition of vascular wall (Higashi et al. 2017). As extra-cardiac signs, vacuolar formation in peripheral leukocytes called Jordans' anomaly can be observed particularly in primary TGCV. In idiopathic one, such vacuolar formation is minor in size but can sometimes be detected.

11.2.4 Postmortem Analysis

Postmortem analysis of cases with idiopathic TGCV showed the following characteristics (Ikeda et al. 2014a, b). (1) TGCV phenotype was frequently observed among autopsied diabetics who died of intractable cardiovascular diseases. (2) Their hearts were heavy in weight with frequent myocardial infarction and hypertrophy. Coronary arteries showed diffuse and concentric stenotic lesions at multivessels. (3) Immunoreactivities of ATGL were detectable in both myocardium and coronary arteries. (4) Imaging mass spectrometry showed that TG deposition was detected in the media and fibrous cap of plaques of coronary atherosclerotic lesions (Fig. 11.2C and red color in Fig. 11.2D). TG signals were overlapped with SMCs.

11.2.5 Genetic Testing

For the diagnosis of primary TGCV, genetic test including sequencing analysis of *PNPLA2* encoding ATGL is obviously useful. In Japan, five mutations have been reported so far. To investigate genetic causes or backgrounds for idiopathic TGCV is an interesting research focus.

11.2.6 Diagnostic Criteria for TGCV and Differential Diagnosis

The Japan TGCV study group provided the diagnostic criteria for TGCV, as shown in Table 11.1. The criteria include two major items (two points each) and two minor items (one point each). Four points or more and three points indicated definite and probable TGCV, respectively.

Here, we can provide the following clinical hints which may suspect TGCV: (1) heart failure that is poorly responsive to beta-blockers; (2) angina pectoris with chest pain that is resistant to nitrates (sometimes 4–5 sublingual tablets of nitroglycerin

Table 11.1 Diagnostic criteria for TGCV

1. Major items (two points)	
1.1 Myocardial TG deposition or impaired metabolism of LCFA	
At least one of the following:	
(1) Myocardial TG deposition by biopsy specimens[a]	
(2) Myocardial TG deposition by MR spectroscopy	
(3) Reduced washout rate (less than 10%) of BMIPP	
1.2 Diffuse narrowing coronary arteries demonstrated by CAG, CT angiography	
2. Minor items (one point)	
2.1 Jordans' anomaly (apparent vacuoles (about 1 μm in size) of polymorphonuclear leukocytes in peripheral blood smear)[b]	
2.2 Diabetes mellitus	
Diagnosis of TGCV	
(1) Four points or more	Definite TGCV
	Primary TGCV, if with ATGL gene mutations
	Idiopathic TGCV, if without ATGL gene mutations
(2) Three points	Probable TGCV
	Definite TGCV, if ATGL gene mutation confirmed

[a]For tissue TG content examination, frozen sections with osmium fixation, but not paraffin sections, should be used for prevention of lipid elution

[b]For difficult cases, May-Giemsa staining slides of the peripheral blood smear samples will be evaluated by the Japan TGCV study group

required for relief); and (3) worsening symptoms during fasting, such as in the morning.

It is important to differentiate TGCV from other cardiovascular diseases including dilated cardiomyopathy, hypertrophic cardiomyopathy, dilated-phase hypertrophic cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy. The differential diagnosis of TGCV also includes the following myocardial diseases, especially lipid storage metabolic diseases: (1) alcoholic heart disease, (2) neuromuscular myocardial disorders, (3) nutritional heart disease, and (4) metabolic myocardial disorders (e.g., Fabry disease, Pompe disease).

Furthermore, the conceptual difference between TGCV and other diabetic and metabolic heart diseases should be noted. Some known disease concepts need to be differentiated from TGCV. One is diabetic cardiomyopathy, which was originally defined as cardiomyopathy without significant stenosis in epicardial coronary arteries. Another concept is fat accumulation in epicardial adipose tissue, which is the overdeposition of TG in physiological tissue. TGCV is distinct from these two entities, because TGCV is characterized by the ectopic deposition of TG in the cardiomyovascular system with apparent involvement of epicardial coronary arteries.

11.2.7 Global Distribution of Patients with Genetic ATGL Deficiency

Here we review the literature (Hirano et al. 2008; Fischer et al. 2007; Pennisi et al. 2017; Kaneko et al. 2014; Chen et al. 2010; Reilich et al. 2011; Muggenthaler et al. 2016; Coassin et al. 2010) regarding patients with genetic ATGL deficiencies (homozygous mutation) published in recent decades. Of the 46 patients with genetic ATGL deficiencies (Fig. 11.3), there are 29 patients without cardiac symptoms and 17 patients with cardiac symptoms (TGCV phenotype) such as dilated or hypertrophic cardiomyopathy-like abnormalities and ventricular tachycardia.

Seven patients with primary TGCV have been so far identified in Japan. Four patients suffered from severe cardiac dysfunction that resulted in cardiac death, and two patients had to undergo heart transplantation due to dilated cardiomyopathy and heart failure (Higashi et al. 2015).

The concept of TGCV is relatively new, and physicians and medical staffs should pay more attention to this kind of disease when they encounter patients who suffer from congestive heart failure and/or coronary heart disease of unknown cause. A definitive diagnosis is essential for treatment and assessment of prognosis. Because cardiac involvement (TGCV phenotype) is critical for patients with ATGL deficiency, delayed diagnosis needs to be avoided. If all patients could be registered in the international registry (http://www.tgcv.org/r/home.html), physicians and medical staffs could share their experience with diagnosis and treatment to reduce patients' difficulties and disabling at earlier stages.

11.3 Biochemical Perspectives

TGCV is characterized by the ectopic accumulation of TG in cardiomyocytes and vascular SMCs that in turn causes pathological conditions such as severe heart failure, coronary artery disease, and arrhythmias (Fig. 11.4) (Hirano et al. 2008, 2014; Hirano 2009). Abnormal metabolism of cellular LCFAs resulting from ATGL deficiency induces both lipotoxicity and energy failure in cardiomyocytes, and these cause HF and coronary artery disease. ATGL is the enzyme that catalyzes the rate-limiting step of the hydrolysis of cellular (cytoplasmic) TG to release the first nonesterified LCFA (Zimmermann et al. 2004, 2009; Haemmerle et al. 2006).

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 mitochondria and undergo β-oxidation to produce ATP, or temporarily become TG, are promptly hydrolyzed by an enzyme such as ATGL, and are transported to the mitochondria to undergo β-oxidation (Fig. 11.5A). Patients with TGCV have abnormalities in ATGL and other components of the intracellular TG hydrolysis pathway that cause intracellular accumulation of triglycerides as lipid droplets (Hirano

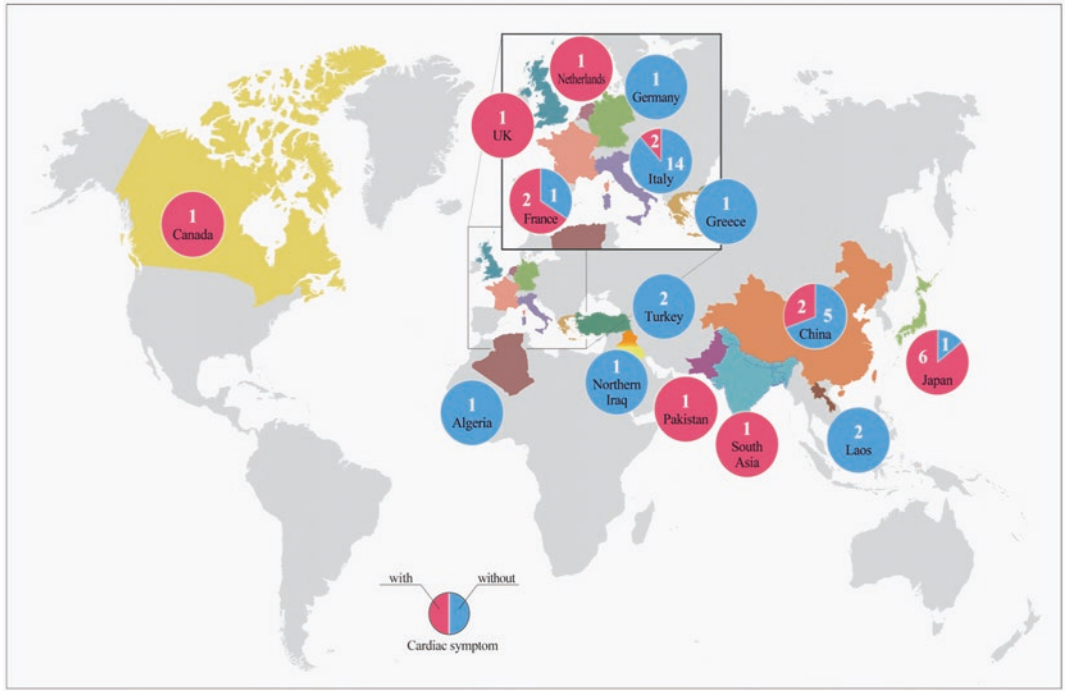

Fig. 11.3 Global distribution of patients with genetic ATGL deficiency Cases of genetic ATGL deficiency without cardiac symptoms are shown in blue, and cases with cardiac symptom (TGCV phenotype) are shown in red

et al. 2014, 2015). Lipotoxicity from intracellular TG accumulation and energy failure caused by the inability of cells to use this energy source are involved in the pathogenesis of TGCV (Fig. 11.5B).

11.4 Therapy

TGCV is resistant to standard medical treatments for HF, angina pectoris, or arrhythmia, including vasodilator, nitrates, and beta-blockers. Two patients with primary TGCV received CTx in Japan. The long-term follow-up indicates that the donor hearts can be maintained under the standard immunosuppression. However, it is noted that, even after CTx, skeletal myopathy develops gradually. Some patients with idiopathic TGCV suffer from slow-flow and no-reflow after PCI, requiring emergent CABG, as described in Case 2. The Japan TGCV study group has started physician-initiated clinical trials for primary and idiopathic TGCV patients to test efficacy and safety of nutritional therapies with medium-chain fatty acids (ClinicalTrial.gov: NCT02830763; NCT02502578).

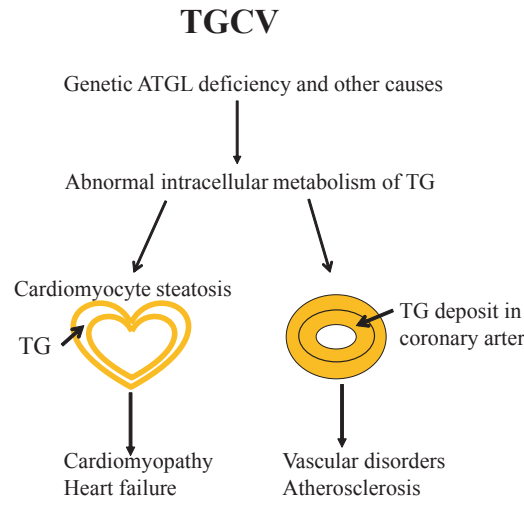

Fig. 11.4 Schematic presentation of the disease concept for TGCV. Genetic ATGL deficiency (primary TGCV) and other causes (idiopathic TGCV) induced cardiomyocyte steatosis and TG deposition of smooth muscle cells, leading to severe heart failure and TG-deposit atherosclerosis

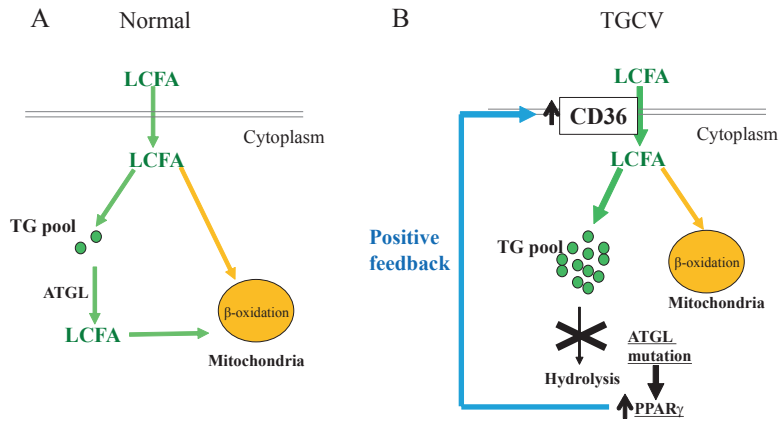

Fig. 11.5 Pathophysiological model for TGCV. Under normal conditions (left panel), LCFAs are taken up through LCFA transporters and receptors such as CD36. Some are transported to mitochondria for beta-oxidation and the remaining LCFAs are utilized as a source of TG and rapidly hydrolyzed by intracellular lipases such as ATGL. In TGCV (right panel), LCFAs are taken up and used to synthesize of TG that cannot be hydrolyzed due to ATGL insufficiency such as genetic mutations, leading to massive TG accumulation. Peroxisome proliferator-activated receptor γ and related genes are upregulated, which induces a positive feedback mechanism that increases the uptake of LCFAs despite the massive deposition of TG

Acknowledgments This was partially supported by research grants for rare and intractable diseases from the Japan Agency of Medical Research and Development (AMED) (Grant No. 17ek0109092h0003) and from the Ministry of Health, Labour, and Welfare of Japan.

Questions

1. Genetic ATGL deficiency is an extremely rare disease, and information is very limited. What is the best approach for summarizing information from case studies and educating healthcare providers?
2. How can TGCV be differentiated from other cardiovascular diseases?

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