O-3 Neutral lipid storage disease with myopathy in a Chinese family

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Objective To study the clinical, myopathological features in neutral lipid storage disease with myopathy (NLSDM) caused by a novel PNPLA2 mutation. **Methods** The two patients are siblings. The proband is a 40-year-old woman. She presented progressive limb weakness and muscle atrophy at 35 years of age. Her 55 year-old brother presented deafness at the age of 35 years old and limb weakness at 45 years old. He has ventricular septal defect. Open biopsies were performed on them and specimens were studied histologically, enzymhistochemically, ultrastructurally. PNPLA2 gene were analyzed in the both patients and three healthy family individuals. **Results** Muscle biopsy in both patients revealed hypertrophy and atrophy of fibers with proliferation of connective tissue. There were numerous lipid droplets and plenty of rimmed vacuoles in the fibers. Electron microscopy revealed lipid droplets as well as myelin figures in the muscle fibers. A single homozygous base substitution were detected at the beginning of intron 2 (IVS2+1G>A) of PNPLA2 in two patients, but not in the healthy family individuals. **Conclusion** The novel IVS2+1G>A mutation of PNPLA2 caused NLSDM with prominent limb weakness, auditory nerve lesion and congenital heart disease. The rimmed vacuoles can appeared in the muscle in this disease.

O-4 Neutral lipid storage disease with myopathy – pathologically a unique entity among lipid storage myopathies

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Lipid storage myopathy is a condition diagnosed by its characteristic finding on muscle pathology – increased lipid droplets in size and number in muscle fiber cytoplasm. There are only four genetically diagnosable entities: primary carnitine deficiency, multiple acyl-CoA dehydrogenase deficiency, neutral lipid storage disease with ichthyosis (NLSDI) and neutral lipid storage disease with myopathy (NLSDM).

In our study, mutations were identified in known causative genes only in 1/4 of patients, indicating that cause is still unknown in majority of cases. So far, we have found two patients with NLSDM.

Both of our NLSDM patients had more remarkable fiber size variation than other lipid storage myopathies. Furthermore, both cases had rimmed vacuoles in muscle fibers, in addition to increased lipid droplets. In contrast, none of patients with other subtypes of lipid storage myopathy has rimmed vacuoles, suggesting that myodegenerative process and its associated autophagic phenomena, similar to rimmed vacuolar myopathy or inclusion body myopathy, may be a characteristic pathological finding of NSLDM.

In my talk, I will review clinicopathological features of all four genetically diagnosable lipid storage myopathies, focusing on NLSDM.

O-5 Neutral lipid storage myopathy may respond to beta-adrenergic treatment

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Objective: Neutral lipid storage disease is caused by mutations in the *CGI-58* or the *PNPLA2* genes. Lipid storage can be detected in various cell types including blood granulocytes. While *CGI-58* mutations are associated with Chanarin-Dorfman syndrome, a condition characterized by lipid storage and skin involvement (ichthyosis), mutations in the patatin-like phospholipase domain-containing protein 2 (*PNPLA2*) were reported with skeletal and cardiac muscle disease only. **Methods**: We describe in detail clinical, myopathological and MRI findings of 6 patients with different recessive *PNPLA2* mutations. Pulse-chase labeling of control and patient cells with supplementation of clenbuterol, salmeterol and dexamethasone was performed *in vitro*. **Results:** The patients share a recognizable clinical phenotype with prominent shoulder girdle weakness, mild pelvic girdle and distal muscle weakness with highly elevated CK and cardiomyopathy developing at later stages. Muscle histology invariably reveals massive accumulation of lipid droplets. New muscle or whole-body MRI techniques may assist diagnosis and may become a useful tool to quantify intramuscular lipid storage. Activation of hormone-sensitive lipase by beta-adrenergic substances such as clenbuterol appears to bypass the enzymatic block in PNPLA2-deficient patient cells *in vitro*. **Conclusions:** PNPLA2 deficiency is a slowly progressive myopathy with onset around the third decade. The diagnosis can be made by staining for Jordans' anomaly in peripheral blood smear. Cardiac involvement is relatively common at a later stage. Muscle MRI may detect increased lipid in a characteristic distribution, which could be used for monitoring disease progression. Beta-adrenergic agents may be beneficial in improving triacylglycerol breakdown in patients with *PNPLA2* mutations.

O-6 RNA Interference-directed Knockdown of Adipose Triglyceride Lipase Enhanced TNFα-induced ICAM-1 Expression in Human Aortic Endothelial Cells via PKC-dependent activation of NF-κB

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Mutations in the human adipose triglyceride lipase (ATGL) gene are associated with neutral lipid storage disease with myopathy, and its cardiac phenotype, known as triglyceride deposit cardiomyovasculopathy (TGCV), shows massive TG accumulation in both coronary atherosclerotic lesions and the myocardium. Recent reports show that myocardial TG content is significantly higher in patients with prediabetes or diabetes, and that ATGL expression is decreased in the obese insulin-resistant state.

We investigated the effect of decreased ATGL activity on the development of atherosclerosis using human aortic endothelial cells (HAECs). We found that ATGL knockdown enhanced monocyte adhesion via increased expression of TNFα-induced ICAM-1. Next, we determined the pathways (Mitogen-activated protein kinase, PKC, or NF-κB) involved in ICAM-1 upregulation. Both phosphorylation of PKC and degradation of IkBa were increased in ATGL knockdown HAECs. In addition, intracellular diacylglycerol levels and free fatty acid uptake via CD36 were significantly increased in these cells. Inhibition of the PKC pathway using calphostin C and GF109203X suppressed TNFα-induced ICAM-1 expression.

These results suggest that reducing ATGL expression may influence the atherogenic process not only in TGCV but in the insulin-resistant state.

P-1 Siblings with Lipid Deposition in Cardiomyocytes

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We report siblings who had similar findings on both optical and electron microscopes.

Patient was 53 years old female. She was on dialysis for about 20 years and decreased heart function was mentioned. Blood pressure was 110/80 mmHg, pulse rate was 77 per minute, and weak systolic heart murmur was audible. Liver enzyme, creatine kinase, and serum creatinine were elevated above normal range. Electrocardiogram showed high QRS voltage and hypertrophic ST-T change. In echocardiogram, left ventricular end-diastolic diameter was 50 mm, and ejection fraction was 27 %. In an optical microscope, disarray of cardiomyocyte with fibrosis was observed, and vacuolar degeneration of cardiomyocyte was also observed with high magnification. Amyloid deposition was not detected. Various size of lipid droplet was observed in a cardiomyocyte by an electron microscope. Neither adipose triglyceride lipase gene nor comparative gene identification-58 gene was detected from her blood sample.

Her three children were all negative for both genes. Her younger brother died at age of 28 of heart failure. He was diagnosed as dilated cardiomyopathy before he died. Autopsy of his heart showed very similar optical and electron microscopic findings with his sister.

P-2 Characteristic feature with infiltration of less intimal macrophage foam cells of arteriosclerotic lesions in triglyceride deposit cardiomyovasculopathy (TGCV)

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Background: We recently reported an unusual case with adipose triglyceride lipase (ATGL) mutation as triglyceride deposit cardiomyovasculopathy (TGCV).

Objectives: This study sought to describe the characteristic feature with inflammatory macrophages of arteriosclerotic lesion in TGCV.

Method: We studied coronary artery samples from explanted heart of the patient with TGCV and compared the findings with classic atherosclerotic lesion of ischemic heart disease (IHD). For histology, sections were immunostained with antibodies directed against CD45RO, CD4, CD8, CD68, CD36 and macrophage scavenger receptor (MSR) on paraffin sections.

We evaluated distribution of inflammatory cells and the ratio of macrophages (M) / [(M) + macrophage foam cells (MF)] on 4 segments of transverse section of coronary artery.

Result: Coronary artery in TGCV showed concentric lesion characterized by TG-accumulated smooth muscle cells in intima and media of the arterial wall. Whereas majority of macrophages predominantly infiltrate in the inner side in contrast to macrophage foam cells in the outer side of the intima. M/ (M+MF) ratio were significantly higher in TGCV than in IHD (60.2% vs. 35.2%, p=0.0303828).

Conclusion: There is arteriosclerotic lesion with infiltration of less intimal macrophage foam cells in TGCV.

P-3 Raman microscopy analysis of triglycerides deposit cardiomyovasculopathy

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An important issue for diagnosis of triglyceride deposit cardiomyovasculopathy (TGCV) is the discrimination of triglyceride from cholesterol ester in tissue, because both are lipid and are stained with typical lipid dyes. We observed Raman spectral images of TGCV patient's myocardium tissue and ICM (ischemic myocardiopathy) patient's coronary artery tissue to distinguish between triglyceride and cholesterol ester. Raman microscopy is a powerful and attractive method of non-labeled molecular imaging. Raman scattering that originates from normal mode of molecular vibrations and its spectrum is sensitive to molecular structure. The Raman images of both samples at 1440 cm^{-1}, which is a band of lipid alkyl chain, were similar to the oil-red-O stained images. On the other hands, Raman signal at 701 cm^{-1}, which is the band of cholesterol, was obtained only in ICM sample and its image was similar to the Raman image at 1440 cm^{-1}. As a result, it was shown that those two Raman bands were marker bands to distinguish triglyceride from cholesterol ester for diagnosis of TGCV with Raman microscopy.

P-4 Establishment of the Induction of Vascular Smooth Muscle Cells from the Patient-specific iPS Cells and the fibroblasts

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The patient with primary triglyceride deposit cardiovasculopathy (TGCV) has intracellular ATGL (adipose tissue triglyceride lipase) dysfunctions in muscle cells of heart and artery. Abnormal lipid deposition was evident in the cells, therefore we tried to induce vascular smooth muscle (SM) cells from the patient-specific iPS cells, characterize the phenotype of the cells, and evaluate the abnormal deposition for the diagnosis. We used mouse embryonic stem (ES) cells to confirm the method of the induction for now. The results showed that 0.01nM all-trans retinoic acid treated cells with Elastin substrates extensively expressed SM cell markers, such as alpha-SMA and SMemb compared to non-treated cells. We successfully established the induction of SM cells from ES cells. In the next step, we apply the established method for the patient iPS cells. Additionally, we already tried to make the constructs of adenoviral transfection in order to establish the method of the direct induction of SM cells from patient-fibroblasts. In this presentation, we also show the strategy of direct induction.

P-5 Biomarker Discovery for Triglyceride Deposit Cardiomyovasculopathy Using Proteome and Transcriptome Analysis

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Triglyceride Deposit Cardiomyovasculopathy (TGCV) is characterized by triacylglycerol deposition in multiple tissues. Mutations in ATGL genes are associated with TGCV, although pathogenic mechanism remains unknown. Here we examined proteomic and transcriptomic profiles of fibroblasts derived from patients and disease-model mice to find biomarker for TGCV.

Fibroblasts derived from healthy volunteer and patients were labeled by stable isotope labeling by amino acids in cell culture (SILAC). Protein extracts of labeled cells were mixed, fractionated by one-dimensional gel electrophoresis and fragmented by in-gel trypsin digestion, followed by MS analysis with a linear ion trap-orbitrap instrument. Transcriptome analyses were also performed using Affymetrix GeneChip Array. Differentially expressed genes and proteins were verified by quantitative PCR and Western blot, respectively. Protein extracts from cardiac muscle of wild-type and ATGL KO mice were homogenized and delipidated. Each sample waslabeled with iTRAQ, fractionated with a strong-cation exchange column and quantitatively identified by LC-MS/MS.

Our multiomics analysis identified several biomarker candidates for TGCV, which include proteins involved in triacylglycerol metabolism such as adipophilin, CGI-58 and G0S2. Also a protein called filaggrin, an intermediate filament-associated protein, was upregulated in the fibroblast of TGCV patients. We are currently validating our results and investigating functions of these candidates.

P-6 Triglyceride Deposit Cardiomyovasculopathy Associated with Type 2 Diabetes Mellitus

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Triglyceride deposit cardiomyovasculopathy (TGCV) is a novel clinical entity we first identified in a patient receiving cardiac transplantation in Japan. The concept of TGCV is that TG accumulates in both the coronary arteries and myocardium, resulting in severe heart disease. The first reported patient with TGCV was a homozygote for mutation of the gene encoding adipose triglyceride lipase (ATGL). We speculated that type 2 diabetes mellitus (DM) patients can show TGCV phenotype because diabetic conditions affect TG metabolism and induce intracellular TG deposition. In this study, we examined autopsy specimens from diabetic subjects for TGCV phenotype.

P-7 Relationship between lipid deposition and ATGL expression in human myocardial tissue: A study with autopsy cases

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Abnormal lipid accumulation in primary triglyceride deposit cardiovasculopathy (TGCV) implies ATGL (adipose tissue triglyceride lipase) plays a role in myocardial lipid metabolism. However, ATGL function in the disease process has not been clarified in the non-primary TGCV individuals. We tested the relationship between lipid deposition and ATGL immunoreactivity in the myocardial tissue using 55 serial autopsy cases. Lipid deposition was detected by Nile blue staining in 3 cases (5.6%) and all positive cases had been suffered from severe Diabetes mellitus (DM) and infectious diseases. Prior to the ATGL staining, we screened specimens by immune-reactivity against desmin and excluded negative 2 cases as inappropriate materials. ATGL immunoreactivity was tested with 3 different antibodies and more than 90% cases were positive against at least one antibody. All 3 cases showing myocardial lipid deposition revealed positive for ATGL staining, suggesting that lipid accumulation was possibly recognized independently with ATGL immunoreactivity. As all severe DM cases did not display lipid deposition in the myocardium, additional insults at the agonal stage such as septic status may be involved in the lipid accumulating process. Lipid deposition in the myocardium may be related enzymatic function of ATGL, otherwise ATGL-independent mechanism in the non-primary TGCV individuals.

P-8 Adipose triglyceride lipase and comparative gene identification-58 are downregulated in the heart of diabetic fatty db/db mice

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Triglyceride (TG) deposit cardiomyovasculopathy shows massive TG accumulation in both coronary atherosclerotic lesions and the myocardium. Recent reports show that myocardial TG content is significantly higher in patients with prediabetes or diabetes and is associated with impaired left ventricular diastolic function. Therefore, we investigated roles of adipose triglyceride lipase (ATGL) and comparative gene identification-58 (CGI-58) in the development of myocardial steatosis in the diabetic state.

TG contents in the hearts of db/db mice, a rodent model of type 2 diabetes, are higher than in those of control mice. Histological examinations assessed with oil red O staining showed marked lipid deposition within the hearts of db/db mice. Next, we determined expression of genes and proteins that affect TG metabolism and found that ATGL and CGI-58 expression were decreased in the hearts of db/db mice. Also, we found increased expression of genes that affect triglyceride synthesis (SREBP1c, monoacylglycerol acyltransferases and diacylglycerol acyltransferases). In addition, we analyzed the expression levels of key modulators of apoptosis and found that Bcl-2 levels were lower in diabetic hearts. These results suggest that reduced ATGL and CGI-58 expression with increased TG synthesis may enhance myocardial steatosis, leading to cardiac apoptosis in the diabetic state.

P-9 A Dietary Therapy with Medium Chain Triglyceride (MCT) for Triglyceride Deposit Cardiomyovasculopathy

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Long chain fatty acid (LCFA) is a major energy source for normal heart. Because we found that LCFA induced lipotoxicity, whereas medium chain fatty acids rescued from cell death in fibroblasts with triglyceride deposit cardiomyovasculopathy (TGCV), we have developed a specific dietary therapy using MCT. The diet consists of 1,800 kcal/day, 45 g of fat, and 70 g of protein. Fat consisted of 15 g natural fat (of which majority is LCFA) and 30 g MCT oil. We have tested their effect in two patients with TGCV. In the first case with 50-day-treatment before cardiac transplantation (CTx), myocardial TG content was reduced in the specimens obtained at CTx compared with those obtained when a left ventricular assist device was implanted three years earlier. In the second case with severe skeletal myopathy, breathing capacity and grasping power was increased after 3-month therapy, indicated that muscle weakness may be partially improved. These results suggested that the developed therapy might be effective in TGCV.

P-10 Effects of medium-chain triglyceride-containing diet on adipose triglyceride lipase KO mice

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[Purpose] We developed a possible diet therapy using medium-chain triglyceride (MCT) for triglyceride deposit cardiomyovasculopathy (TGCV) at Osaka University Hospital. The present study verified MCT diet therapy efficacy by examining effects of a MCT-containing diet (MCT diet) on life span, myocardial fat accumulation, and male fertility in adipose triglyceride lipase (ATGL) KO mice.

[Methods] ATGL KO mice were divided into 2 groups and fed a control or MCT diet at weaning (4 weeks). CT value as an index of myocardial fat accumulation and left ventricular ejection fraction (EF) were determined by experimental animal CT imaging (Hitachi-Aloka Medical, Ltd.). Isolated heart was examined histologically, and sperm motility was examined.

[Results] Life span was prolonged significantly with the MCT diet versus the control diet. Myocardial CT value and EF were improved with the MCT diet versus the control diet. Histological examination showed inhibition of myocardial cell fat accumulation in the MCT diet group. Sperm motility, scarcely observed in the ATGL KO mice, was improved with the MCT diet.

[Conclusion] Prolonged life span, inhibition of myocardial fat accumulation, inhibition of exacerbation of cardiac functions, and fertility improvement were observed in the ATGL KO mice fed the MCT diet. Proof of concept was conducted for implementation of MCT diet therapy clinical studies.

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P-11 New approach for viral myocarditis by MCT diet

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The hypothesis that viral myocarditis causes an autoimmune response and subsequent DCM is controversial. We have already demonstrated that repetitive coxsackievirus B3 (CVB3) infection may cause cardiac dysfunction and dilation without inflammatory cells infiltration in mice. There are no clinical evidence for the cardioprotective effects of any drugs in the acute myocariditis. To determine critical role of MCT diet for development of viral myocarditis, a mouse model of coxsackievirus B3 (CVB3) induced myocarditis was used.

Methods: Threee-week old A/J male mice were inoculated with CVB3 2×10^4 PFU, intraperitoneally. Ten mice were feeded with 5% MCT or LCT for 5 days before and 14 days after CVB3 inoculation. All mice were sacrified at day 14 after the viral inoculation. **Results:** We demonstrated that the cardioprotective effects of MCT diet in the acute setting of viral myocarditis which have been experimentally demonstrated in mice. Exploring the nature of the lipid deposition found in the myocardium in this study will provide further insights into the metabolism of viral myocariditis.

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これまでの原発性 TGCV (ATGL 欠損症)の報告例

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◆厚生労働省◆
難治性疾患克服研究事業 研究班
からのお願いです。

日本小児循環器学会
会員各位

2008年、わが国の心臓移植症例より、細胞内中性脂肪分解酵素欠損により心症状及びミオパチーを呈する新規疾患単位、中性脂肪蓄積心筋血管症（Triglyceride Deposit Cardiomyovasculopathy, TGCV)が、同定されました。現在のところ成人例しか同定されていませんが、発症から数年～10数年で、重症心不全を呈すると考えられるため、小児期での診断が重要と考えられます。

そこで会員の皆様に本疾患の概念・診断についてお知らせいたします。
もし、本疾患が疑わしい患者さんをご存じでしたら

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まで、ご連絡いただければ幸いです。宜しくお願い申し上げます。

平成24年3月

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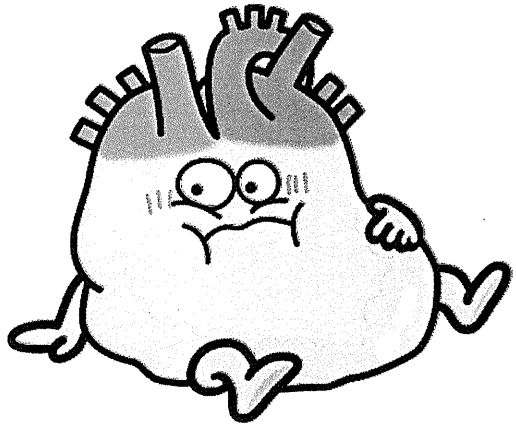
大阪大学医学部附属病院　循環器内科　平野賢一

“中性脂肪蓄積心筋血管症”
Triglyceride deposit cardiomyovasculopathy

(Hirano K, et al. N Engl J Med. 2008)

詳細は 裏面を
ご覧ください。



新しい疾患：
中性脂肪蓄積心筋血管症
Triglyceride Deposit Cardiomyovasculopathy (TGCV)
-知識の普及および早期発見に向けて-

＜疾患の紹介＞

2008年、我が国の心臓移植症例から見出された新規疾患であり（Hirano K, *et al.* N Engl J Med. 359: 2396-2398, 2008)、心筋、冠状動脈に中性脂肪が蓄積することにより心不全、不整脈、虚血性心疾患などを呈する。心筋症と診断されるケースが多い。20歳代から中年にかけて発症し数年から数十年で死にいたる難病。心外症状として骨格筋ミオパチーを呈するが症例によりその程度は異なる。現在のところ明らかな原因は、細胞内中性脂肪分解酵素 adipose triglyceride lipase (ATGL)の遺伝的欠損である。これまで、国内7症例（4例は20-50才代で心臓死、2例は、心臓移植）、国外16症例が見出されている。これまで報告された最若年例は、米国の18才女性で、心筋、骨格筋に中性脂肪蓄積を認めるものの心症状、骨格筋症状を認めない（Akman HO, *et al.* Neuromuscul Disord 20: 397-402, 2010)。

＜早期発見の必要性＞

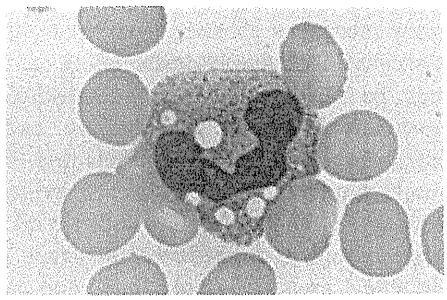
上述のように20歳を過ぎると発症する可能性が出てくること、発症した時点でかなり進行している症例もいること、発症後、数年～10年数年で死に至ることより、10歳代のうちに発見されるべきである。

＜検査所見＞　成人では以下の所見が認められる。

1．末梢血塗抹標本にて、多核白血球、単球に著明な空胞変性（下図：Jordans' anomaly と呼ぶメイギムザ染色）が全症例で見られている。幼少時から見られるものであろうと推測される。

2．組織における中性脂肪の蓄積にも関わらず、血清中性脂肪値、カルニチンレベルなどは正常者と同様な値である。CPKが高値を示す症例はある。

3．研究班では、簡易スクリーニング法の開発バイオマーカーの探索に成功しております。ご興味のある先生方は、平野までご連絡をお願い申し上げます。



＜治療＞

1．2症例に心臓移植が施行されている。

2．2症例に対して中鎖脂肪酸を含む食事療法が、大阪大学医学部附属病院において自主臨床研究として行われ、有効性が示唆されている。本療法の効果は、ATGL KOマウスにおいても確認された。酵素補充療法が理想的で、開発を促すため早期診断、症例数の把握が重要である。

ご協力のほど、何卒よろしくお願い申し上げます。

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portable cautery device, which may not be available in a primary care office. Instead, the time-honored approach is to heat the end of an unfolded paper clip in a flame and to use that metal to burn through the nail into the hematoma.

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THE AUTHORS REPLY: Although we recognize, as Trautinger points out, that the pathobiology of sunburns may not be typical of most thermal burns, one of the most common causes of first-degree burns is sunburn. Thus, topical diclofenac sodium may be of use for many patients with first-degree burns. There is also evidence that other topical nonsteroidal antiinflammatory agents may be of benefit for thermal injuries. For example, a study of second-degree burns in adult sheep showed that topically applied ibuprofen decreased both local edema and prostanoid production in the burn tissue.[1]

We agree that all efforts should be made to reduce the risk of bacterial cross-contamination between patients and wounds. Previous studies have shown that white-coat sleeves often contain pathogenic bacteria such as *Staphylococcus aureus*.[2,3] In these studies, a significant proportion of subjects laundered their coats only at monthly intervals. No study has shown contamination of white coats that were properly washed and changed on a daily basis. However, we agree with Guyot et al. that the use of clean short sleeves, as well as proper hand washing and gloves, should be encouraged.

Finally, as noted by Kaufman, in the absence of a portable cautery device, the end of an unfolded paper clip, heated in a flame, may be used to drain a subungual hematoma. Although we too have used this method in the past, in our experience it is now often difficult to find an alcohol lamp, let alone a match to light it.

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Triglyceride Deposit Cardiomyovasculopathy

TO THE EDITOR: A 41-year-old man was admitted to our hospital with ventricular tachycardia in 2003. Biopsy specimens obtained from the right ventricle showed neutral lipid deposition in cardiomyocytes. In 2004, the patient had catecholamine-dependent congestive heart failure, and a left ventricular assist system was implanted. Skeletal-muscle atrophy in the arms became evident, and staining of biopsy specimens with oil red O showed droplets of predominantly type I fibers (Fig. 1A). Levels of plasma lipids and carnitine were normal.

In June 2007, the patient underwent cardiac transplantation. Microscopical examination of the explanted heart revealed numerous vacuoles that stained positive for oil red O in the cytoplasm of cardiomyocytes (Fig. 1B). The triglyceride content in the left ventricles was markedly increased, as compared with that in three control subjects without heart disease (data not shown). The patient's coronary arteries showed diffuse intimal thickening and fibroatheromatous lesions. Vacuoles were observed in the cytoplasm of endothelial cells, in the smooth-muscle cells in the media of the coronary arteries (Fig. 1C), and in the foam cells in the intima. Cells that were positive for oil red O staining were seen in the endothelium, intima (Fig. 1C, upper inset), and media (Fig. 1C, lower inset). Surprisingly, the triglyceride content (Fig. 1D), but not the cholesterol content (Fig. 1E), in the patient's atherosclerotic coronary arteries was much higher than that in two control subjects and in two patients with ischemic cardiomyopathy.

To determine the molecular mechanism for this triglyceride deposition, we sequenced the adipose triglyceride lipase gene (*ATGL*, also known as *PNPLA2*), which encodes an essential intracellular triglyceride lipase.[1] The patient was homozygous for a point mutation in exon 7 of *ATGL* (c.865C→T; p.Gln289X), which is identical to a mutation re-

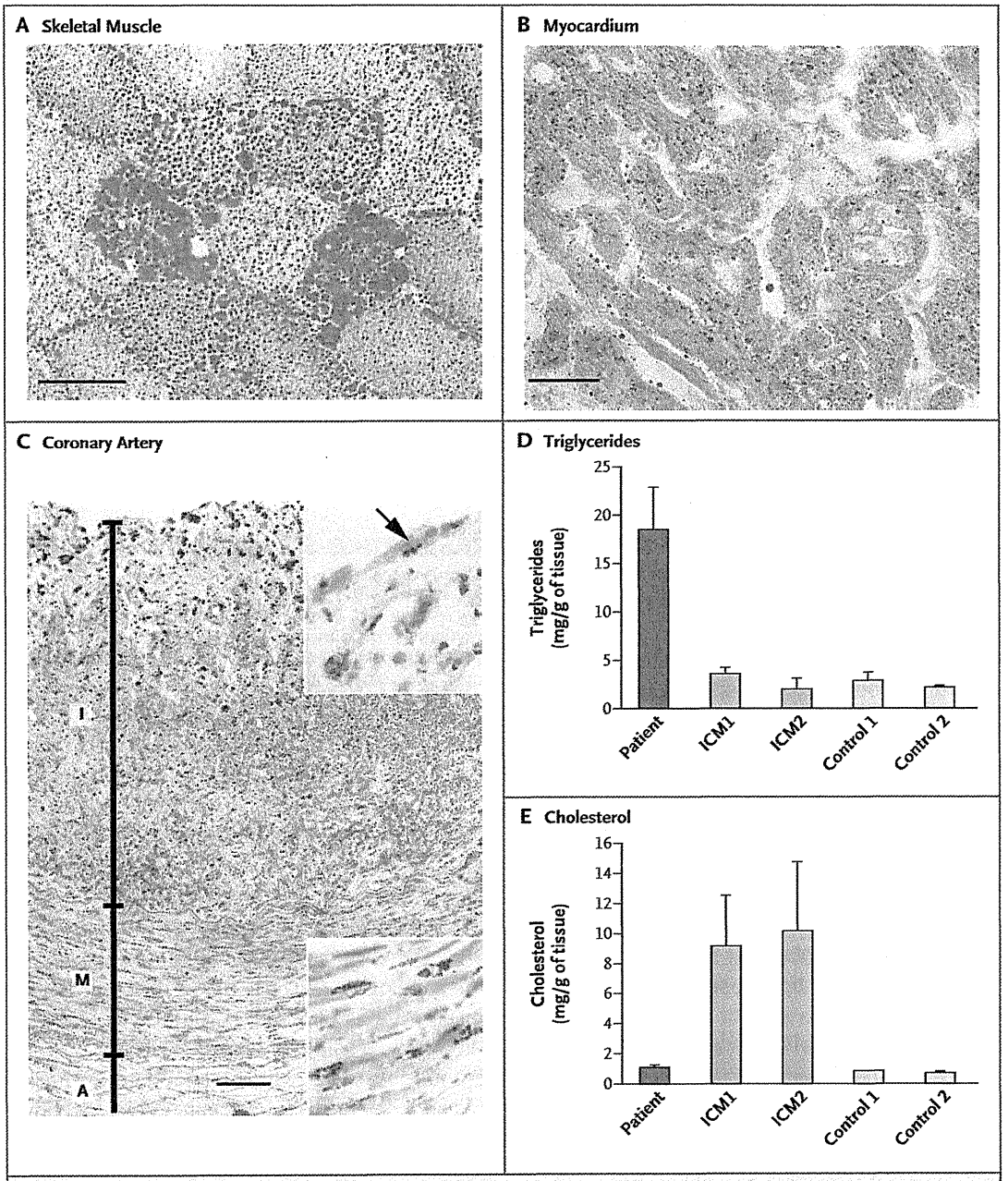

Figure 1. **Oil Red O Staining of Biopsy Specimens from the Patient and Measures of Triglycerides and Cholesterol.** Panel A shows a biopsy specimen obtained from the patient's atrophied arm, with droplets of predominantly type I fibers. The scale bar represents 40 μm. Panel B shows numerous vacuoles in the cytoplasm of cardiomyocytes in the myocardium of the explanted heart after the patient underwent cardiac transplantation. The scale bar represents 50 μm. Panel C shows a histologic section of a coronary artery from the explanted heart. Neutral lipids have accumulated in the endothelium, intima (I), and media (M). The insets show endothelial cells (upper inset, arrow) and smooth-muscle cells (lower inset) in the media that are filled with stained lipid. The scale bar represents 50 μm. The letter A denotes adventitia. Also shown are the triglyceride content (Panel D) and cholesterol content (Panel E) of coronary-artery specimens from the patient, from two subjects with ischemic cardiomyopathy (ICM1 and ICM2), and from two control subjects who died from noncardiac diseases. The T bars represent standard deviations.

ported by Fischer et al.[2] in a patient with mild myopathy.

The atherosclerotic lesion that we observed in this patient was unusual[3] because the accumulated lipid was triglyceride rather than cholesterol, lipid-laden cells were distributed through all layers of the arterial wall, and the patient had normal plasma triglyceride levels. These phenotypes may result from the mutation in *ATGL*.[4]

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Emergence of Extensive Drug Resistance during Treatment for Multidrug-Resistant Tuberculosis

TO THE EDITOR: We report the development of fluoroquinolone-resistant tuberculosis and extensively drug-resistant tuberculosis during second-line treatment for multidrug-resistant tuberculosis in Karakalpakstan, Uzbekistan. Eighty-seven patients were treated with a regimen containing at least five drugs to which the infecting strain was presumed to be susceptible, according to recommendations from the World Health Organization.[1,2] We performed drug-susceptibility testing and DNA fingerprinting on *Mycobacterium tuberculosis* isolates collected at baseline and during treatment.

None of the 87 patients had ofloxacin resistance at baseline, yet ofloxacin resistance developed during treatment in 18 patients (21%), and 10 patients (11%) were classified as having extensively drug-resistant tuberculosis.[3] Only 5 (28%) of the 18 patients with ofloxacin resistance were successfully treated. Isolates from 13 patients had identical DNA fingerprints throughout treatment, probably reflecting the induction and amplification of ofloxacin resistance. A mixed infection, with two strains at baseline, was found in one patient, whereas the isolates obtained from four patients during treatment had DNA fingerprints that differed from those of the baseline isolates, indicating potential reinfection (Fig. 1).

Among the 13 patients with identical strains at baseline and during treatment, second-line resistance and a severe clinical condition at baseline were significantly associated with the development of ofloxacin resistance on univariate analysis ($P=0.002$ and $P=0.03$, respectively) (see the Supplementary Appendix, available with the full text of this letter at www.nejm.org). Both factors remained significantly associated with fluoroquinolone resistance in a multivariate model ($P=0.007$ and $P=0.03$, respectively). Interestingly, 9 of the 13 patients were infected with a multidrug-resistant tuberculosis clone that is highly prevalent in this region, suggesting a higher propensity of particular strains to acquire resistance. A reduction in population diversity caused by clonal expansion of particular multidrug-resistant strains also renders strain differentiation based on IS*6110* fingerprints more difficult. Thus, some of the presumed amplification might represent reinfection with a fluoroquinolone-resistant variant of the same strain.

This study shows that exogenous reinfection with extensively drug-resistant *M. tuberculosis* strains may occur during second-line treatment of multidrug-resistant tuberculosis. The reinfecting strains from three patients showed DNA fingerprint patterns and resistance profiles that were identical to

Communication

A Novel Clinical Entity: Triglyceride Deposit Cardiomyovasculopathy
— Implications and Perspectives from "Obesity of the Heart"

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Heart diseases, including atherosclerotic cardiovascular disease and congestive heart failure, are major life-threatening disorders in most countries. Cholesterol is a vital causal factor and focus of research into heart diseases, but the involvement of triglycerides remains unclear. We recently reported a unique patient suffering from severe congestive heart failure and needing cardiac transplantation. Massive accumulation of triglycerides was observed in coronary atherosclerotic lesions as well as in the myocardium, while plasma triglyceride levels were normal. We suggested that this phenotype was a novel clinical entity and named it "Triglyceride deposit cardiomyovasculopathy", or simply "Obesity of the heart". The patient was identified as homozygous for a genetic mutation in the adipose triglyceride lipase, an essential molecule for hydrolysis of intracellular triglycerides. The present paper deals with what we can learn from this single case and discusses its implications for research and clinical medicine related to heart diseases.

J Atheroscler Thromb, 2009; 16:702-705.

Key words; Adipose triglyceride lipase, Atherosclerosis, Cardiac transplantation, Congestive heart failure, Triglycerides, Triglyceride deposit cardiomyovasculopathy

Cholesterol has been a principal focus of heart disease research during the last 50 years. The Framingham heart study, which started in 1960, reported that plasma cholesterol levels were a strong predictor for coronary heart disease[1]. In the 1970s, Brown and Goldstein clarified the molecular mechanism for familial hypercholesterolemia (FH) and indentified the low density lipoprotein (LDL) receptor as the molecule responsible for FH[2]. In the same decade, Endo *et al.* discovered fungal metabolites with hypocholesterolemic activities[3], which were the prototype of what are currently called "statins". Recently, clinical studies with "power statins" showed that almost half of all cardiac vascular events and deaths can be prevented with these kinds of drugs[4]. Recent reports seem to indicate that statins may be effective for non-ischemic as well as ischemic congestive heart failure[5].

In contrast to cholesterol, the involvement of triglycerides (TG) in heart diseases has remained ambiguous. In spite of their best efforts, it has been difficult for researchers to obtain direct proof of an association between plasma TG levels and coronary heart disease[6, 7]. Before the Framingham heart study and the aforementioned two major discoveries of LDL receptors and statins, pathologists had reported that the human aorta and coronary arteries contained substantial amounts of TG, comparable to cholesterol[8, 9]; however, it seems that the big cholesterol "wave" swept TG away from the heart to the adipose tissue.

TG is synthesized from glycerol and fatty acids and hydrolyzed by intracellular lipases (i.e., adipose triglyceride lipases (ATGL)) and extracellular lipases (i.e., lipoprotein lipases)[10]. Adipocytes take up long-chain fatty acid (LCFA), synthesize TG, and store them in cytoplasmic lipid droplets as a major energy source for the whole body. When required, TG is hydrolyzed by lipases and LCFA is delivered through the bloodstream to oxidative tissues, such as the heart and some skeletal muscles. The heart, which must beat approximately one hundred thousand times a day, prefers LCFA to produce adenosine triphosphate (ATP) via mitochondrial β-oxidation in order to achieve maxi-

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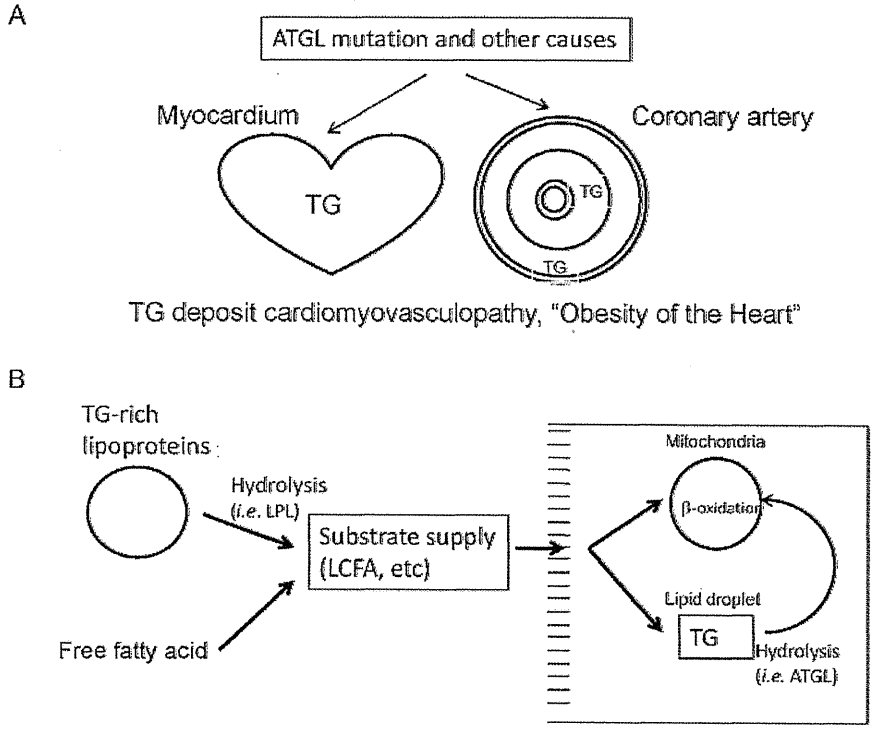

Fig. 1. (A) A schematic presentation of triglyceride deposit cardiomyovasculopathy, showing primary and secondary causes which induce TG accumulation in both myocardium and coronary arteries. (B) Regulatory factors of intracellular TG content.

TG is stored in cytoplasmic lipid droplets. Cellular TG contents may be regulated by catabolism or hydrolysis of intracellular TG as well as by substrate supply, such as LCFA of TG from the bloodstream. LPL: lipoprotein lipase; ATGL: adipose triglyceride lipase; LCFA: long-chain fatty acid.

mum contractility[11]. Under normal conditions, lipid droplets containing TG are therefore hardly observed in the heart.

We recently reported a unique patient suffering from congestive heart failure and eventually needing cardiac transplantation. This patient's coronary arteries and myocardium showed massive TG accumulation even though plasma TG levels were normal. Cardiomyocytes and all layers in the coronary arteries were filled with TG-containing lipid droplets. The patient was identified as homozygous for a genetic mutation in the adipose triglyceride lipase (ATGL), which is an essential molecule for hydrolysis of intracellular TG[12]. We suggested that this phenotype was a novel clinical entity and named it "Triglyceride deposit cardiomyovasculopathy (TGCV)" or simply "Obesity of the Heart" (**Fig. 1A**). It is interesting that the phenotype of TGCV is very similar to that of ATGL knockout mice generated by Zechner *et al.*[13].

What can we learn from this single case of "Obesity of the Heart"?

1. We need to be aware of the presence of a substantial amount of TG in human arteries.

2. It is difficult to differentiate TG from cholesteryl ester by using conventional Oil red O or Sudan IV staining methods. For example, Oil red O-positive lipids in arteries may not always be cholesteryl esters, but rather TG.

3. As mentioned earlier, it seems difficult to prove a direct association between plasma TG levels and coronary heart disease. We need to consider that tissue TG content may depend on intracellular catabolism (hydrolysis) of TG mediated by lipases (ATGL etc) rather than on a supply from substrates such as LCFA from plasma (**Fig. 1B**).

4. The frequency of TGCV phenotype occurrence may be higher in Japan. Since the 1980s, cases of Jordan's anomaly have been reported in Japan in which patients suffered from cardiomyopathy associated with neutral lipid deposition, although their

molecular basis was not identified[14-16]. More recently, it was reported that patients with ATGL mutation had severe heart disease[17, 18], but without mention of TG deposition in coronary arteries.

Possible implications for research and clinical medicine:

1. Elucidation of pathophysiology of TGCV
2. Investigation of the initiation and progression of atherosclerosis in TGCV, particularly focusing on the difference between atherosclerosis in TGCV and classical atherosclerosis, described by Ross *et al.*[19, 20].
3. Determination of the relevance of TGCV phenotype. For this purpose, a population with a high prevalence of TGCV needs to be found.
4. Identification of primary and secondary causes of TGCV
5. Development or laboratory test or other method for easy evaluation of tissue TG accumulation.
6. Examination of the relationship between TG deposition in the heart and acute coronary syndrome.

Medical science has seen novel hypotheses put forward and breakthroughs achieved, such as those for FH[2] and Tangier disease[21], as a result of patient-oriented research[22]. "Cholesterol and atherosclerosis" seem to be close to the goal, whereas "Inflammation and atherosclerosis" are the subject of heated discussion[23]. This single reported case may indicase that this is a suitable time to ask whether "TG" comes back from adipose tissue to the heart.

Acknowledgements

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