脂質プラークの証明には、血管内超音波（IVUS)などが、有用である。

（７）組織学的所見

心筋生検組織、心移植術摘出心ないし剖検心において以下の所見を認める。

肉眼的所見：

特異的な肉眼所見はなく、合併症による修飾を受ける。求心性肥大は高血圧合併例で目立ち、一方、心機能低下例や透析導入症例では心拡大を示す。また冠動脈硬化の進行した例では、瘢痕ないし梗塞像を伴う。冠動脈においては起始部から末梢にかけて、白色調を呈するびまん性求心性肥厚を呈する。

光顕所見：

多くの症例で心筋細胞の肥大と血管周囲性ないし間質性の線維化を示す。あるいは虚血性瘢痕の形成を伴う場合もある。心筋内の中性脂質の沈着がパラフィン包埋切片の HE 染色標本で気付かれる事は稀であり、オイルレッド染色、ナイルブルー染色等の脂肪染色が有用である。細小動脈では内皮細胞下に PAS 陽性物質沈着を伴う硬化像を伴うことが多い。

免疫組織染色： ATGL の免疫原性の消失は見られない。

電顕所見：心筋内の脂肪滴の沈着を検出するのに有用である。
また、心筋細胞におけるミトコンドリアの多形化や数の増加、
T 管の拡大、筋小胞体の拡張、無構造基質の沈着や毛細血管における基底膜の肥厚などが認められる。

質量顕微鏡：
ホルマリン固定標本、凍結標本を用いて、TG の同定を行うことが可能である。

注）組織内脂肪の検出には、オイルレッドO染色などの脂肪染色が有用であるが、凍結切片の利用やオスミウム処理で脂質の溶出を防止するなど特別な検体処理が必要である。

５、 鑑別診断

原発性 TGCV、高血圧性心疾患、肥大型心筋症、拡張型心筋症、拡張相肥大型心筋症、不整脈源性右室心筋症との鑑別が重要である。
さらに、以下の特定心筋症（二次性心筋疾患)、特に蓄積性代謝疾患との鑑別が必要である。

① 原発性 TGCV、②アルコール性心疾患、③産褥性心筋症、④心筋炎
⑤ 神経・筋疾患に伴う心筋疾患、⑥膠原病に伴う心筋疾患、⑦栄養性心疾患、⑧代謝性疾患に伴う心筋疾患（Fabry 病、ヘモクロマトーシス、

Pompe 病など)、⑨アミロイドーシス、サルコイドーシスなど。

6、　臨床経過および予後

一般に予後不良であり、発症から数年から10数年で心不全、虚血性心疾患、脳卒中などで死に至る。既存の治療法に抵抗性で、新たな観点からの治療の開発が強く望まれる。研究班では中鎖脂肪酸 (Medium chain triglyceride, MCT)による食事療法を開発し、臨床研究の準備をしている。

原発性TGCV（ATGL欠損症）の診断

臨床像：　年齢、性、罹患臓器、症状
検査所見：末梢血のJordans' 奇形
心臓CT：　びまん性、貫壁性の低CT値
　ARVC: 心外膜側から、虚血性：　心内膜側から
心筋生検：
　心筋細胞質内の多数の泡沫状の空胞
　　オイルレッドOなど脂肪染色陽性
　　可能ならば、質量顕微鏡などでTGの同定
　DCM, ARVC, HCMなどとの鑑別：抗ATGL抗体が有用
　　上記疾患では、間質細胞、泡沫心筋で陽性になる。
ATGL遺伝子解析→確定診断

参考所見：
　間接熱量測定計：呼吸商
　BMIPPシンチ：　Washout Rate の低下

糖尿病関連TGCVの診断フローチャート

1. 腎症を持つ非肥満の2型糖尿病（日本糖尿病学会の基準による）
   1）持続性タンパク尿、 2) BMIは、26以下
2. 既存の治療法（ライフスタイル改善、内服治療等）にも関わらず、
   コントロール不可群（HbA1c 8.0%以上）。
   （日本糖尿病学会の基準による）
3. 心臓CTにおいて、心筋内の低CT値或いは、心筋生検により、
   心筋細胞が、オイルレッドO染色などで陽性に染色される。
4. 心臓CT或いは冠動脈造影によって、多枝に、びまん性、求心性
   狭窄を認める（偏心性病変の存在の有無は考慮しない）。
5. 質量顕微鏡によって中性脂肪蓄積を証明しえれば、診断価値は高い。

１－５は、必須項目とする。

〔V〕

国際シンポジウム
研究班会議報告

わが国で見出された新規疾患単位

中性脂肪蓄積心筋血管症(TGCV)

の一日も早い克服を目指して

THE FIRST INTERNATIONAL SYMPOSIUM on

Triglyceride Deposit Cardiomyovasculopathy & Neutral Lipid Storage Disease

http://www.cnt-osaka.com/tgcv/sympo2011/sympo2011.html

会期：　2011年11月26日(土)

会場：　京都大学百周年時計台記念館

共催：厚生労働省難治性疾患克服研究事業 中性脂肪蓄積心筋血管症研究班
中性脂肪蓄積心筋血管症研究会

連絡先

大阪大学医学部附属病院循環器内科

平野 賢一

TEL 06-6872-8215(直通)

E-mail khirano@cnt-osaka.com

＊使用しているモチーフは、わが国で見出された最古のATGL欠損症の心臓断面像・Sudan III染色像・多核白血球の電顕像(ご提供：東京都立広尾病院病理 田中道雄先生)。

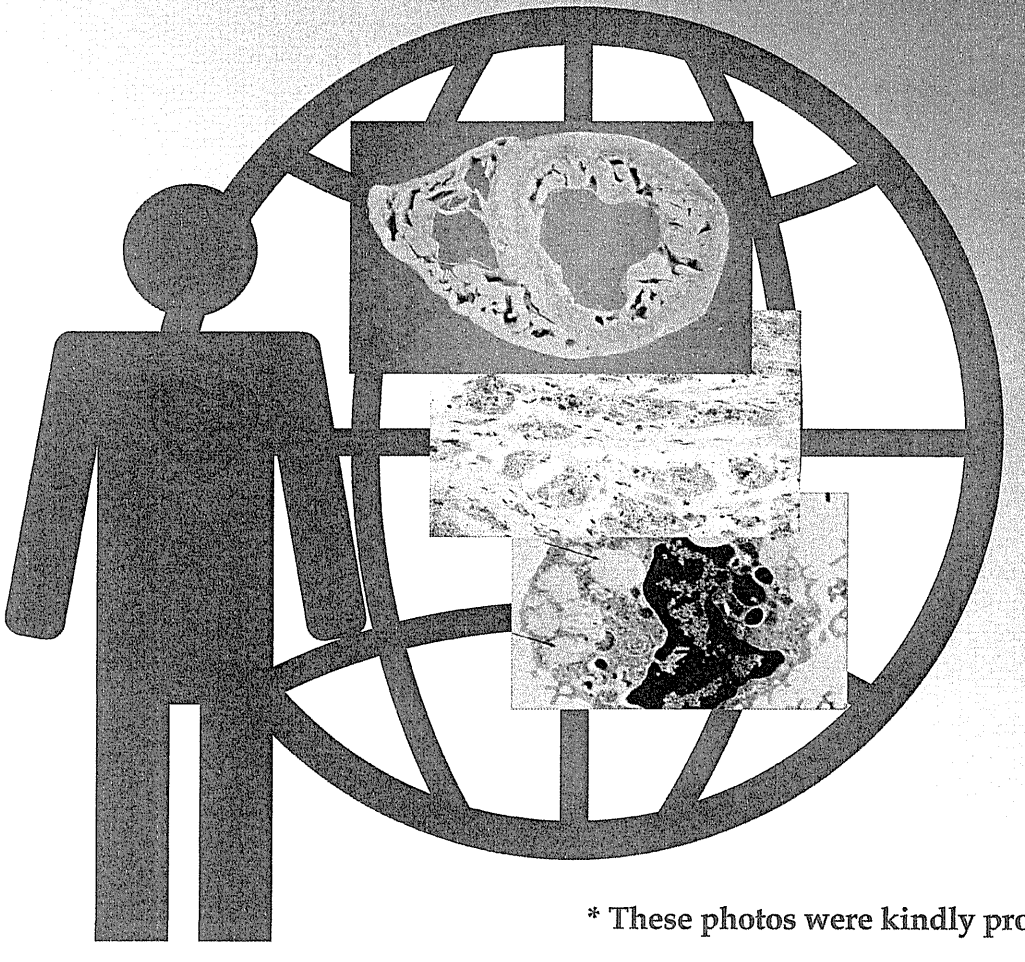
OUR MISSION IS TO
OVERCOME THIS INTRACTABLE DISEASE - TGCV

THE FIRST INTERNATIONAL SYMPOSIUM on

Triglyceride Deposit Cardiomyovasculopathy & Neutral Lipid Storage Disease

PROGRAM & ABSTRACTS

DATE: November 26, 2011
VENUE: Kyoto University Clock Tower Centennial Hall 2F
PRESIDENT: Ken-ichi Hirano Osaka University



* These photos were kindly provided from Dr. Michio Tanaka.

THE FIRST INTERNATIONAL SYMPOSIUM on

Triglyceride Deposit Cardiomyovasculopathy & Neutral Lipid Storage Disease

PROGRAM & ABSTRACTS

DATE: November 26, 2011

VENUE: Kyoto University Clock Tower Centennial Hall

PRESIDENT: Ken-ichi Hirano Osaka University

SPECIAL LECTURE at the 33rd Cardiac Biopsy Conference

Adipose triglyceride lipase: Role in energy metabolism and implications for metabolic disorders

Robert Zimmermann
Institute of Molecular Biosciences, Graz, Austria

Lipolysis, i.e. the catabolism of cellular triacylglycerol (TG) stores, provides free fatty acids (FFA) serving as energy substrate, precursors for other lipids, and lipid signaling molecules. Within the last decade it became evident that the lipolytic pathway is incompletely understood. Hormone-sensitive lipase (HSL) was considered as rate-limiting TG lipase and stayed in the focus of research for three decades. However, the characterization of HSL-deficient mice provided compelling evidence that HSL is not uniquely responsible for the hydrolysis of stored fat. This observation led to the discovery of a novel TG hydrolase named adipose triglyceride lipase (ATGL) and its co-activator protein comparative gene identification 58 (CGI-58). ATGL specifically cleaves the first fatty acid from TG generating diacylglycerol and CGI-58 stimulates its activity manifold. Studies in mutant mice lacking ATGL revealed a defect in lipolysis and severely reduced circulating FFA levels. Fasting of ATGL deficient mice leads to depletion of liver glycogen stores within a few hours and prolonged starvation induces hypoglycemia, reduced energy expenditure, and hypothermia. These observations suggest that ATGL-deficient mice cannot mobilize sufficient energy from TG stores to maintain energy homeostasis under fasting conditions. The reduced availability of FFA is compensated by an increased utilization of carbohydrates for energy conversion.

Loss-of-function mutations in both the ATGL and the CGI-58 gene are associated with TG accumulation multiple tissues in humans and rodents. Yet, the resulting clinical manifestations are not identical. Patients with defective ATGL function suffer from more severe myopathy than patients with defective CGI-58 function, a rare inherited disease named Neutral Lipid Storage Disease with myopathy (NLSD-M). On the other hand, CGI-58 mutations are always associated with ichthyosis, which is not observed in NLSD-M, and this disease was thus named NLSD with ichthyosis (NLSD-I, also known as Chanarin-Dorfman Syndrome). Similar observations were made in mutant mouse models lacking ATGL or CGI-58 indicating a yet unknown ATGL-independent function of CGI-58 in the skin. ATGL-deficiency is associated with massive TG accumulation in cardiomyocytes, cardiomyopathy, and premature death. Recent evidence suggests that the lack of ATGL in cardiomyocytes decreases mRNA levels of PPAR α and PPARδ target genes. This leads to severely impaired mitochondrial substrate oxidation and respiration. Interestingly, pharmacological reconstitution with PPARα agonists reverses mitochochondrial dysfunction, restores normal heart function, and prevents premature death. These findings indicate that PPAR activation requires ATGL-mediated lipolysis and reveal a potential treatment of patients suffering from NLSD.

The rate-limiting role in lipolysis makes ATGL to an interesting pharmacological target since increased lipolysis is linked to at least two unfavorable metabolic conditions: Insulin resistance and cachexia, which is most commonly observed in cancer patients. Notably, mice lacking ATGL exhibit increased insulin sensitivity and glucose tolerance and do not develop high-fat diet-induced type 2 diabetes. Moreover, ATGL-deficient mice are resistant to cancer-associated cachexia implicating that functional lipolysis is essential in the pathogenesis of this disease. Thus, pharmacological inhibition of ATGL could improve insulin sensitivity in diabetic patients and counteract the development of tumor-induced cachexia.

Dear Colleagues:

In 1975, a 22-year-old male was admitted to a hospital because of palpitation. He was diagnosed as cardiomyopathy and ventricular tachycardia with multiple lipid droplets in his peripheral leucocytes called "Jordans' anomaly". A couple of years later, he unfortunately died of progressive heart failure. A pathologist observed massive triglyceride accumulation in his autopsied heart and stored formalin-fixed specimens for future research (please see the cover).

In 2008, we reported a cardiac transplant recipient with massive TG accumulation in his myocardium as well as unusual coronary atherosclerotic lesions. We named this phenotype triglyceride deposit cardiomyovasculopathy (TGCV) (*N Engl J Med.* 2008). In 2009, we launched the Japan TGCV study group supported by the Japanese Government. The known primary cause of TGCV so far is the genetic mutation in the adipose triglyceride lipase (ATGL), an essential molecule for intracellular hydrolysis of TG, which was discovered by Zimmermann et al (*Science.* 2004).

Recently, the patient whom I described at the beginning turned out to be ATGL deficiency more than thirty years after his premature death. To our knowledge, he is the oldest ATGL deficiency ever identified. Therefore, we have decided to use photos of his pathological sections as the motif of our symposium, which was kindly provided by Dr. Michio Tanaka, Tokyo, Japan.

In this symposium, more than fifty scientists from abroad and Japan participated and heatedly discussed about how we diagnose and treat patients. I assure that this is the first important step to construct the international co-operative group to overcome this intractable disease.

Thank you very much.

Ken-ichi Hirano, MD, PhD

November 26, 2011／Kyoto University Clock Tower Centennial Hall

CONTACT: c/o Hirano Laboratory (Rm. #314)
Laboratory for Cardiovascular Disease Novel, Non-invasive, Nutritional Therapeutics
Open Laboratories for Advanced Bioscience and Biotechnology, Osaka University
6-2-3 Furuedai, Suita 565-0874, JAPAN
TEL +81-6-6872-8215 FAX +81-6-6872-8129 E-mail jim@cnt-osaka.com

PROGRAM & CONTENTS

Oral Session 1

Chairpersons: Masatsugu Hori (Osaka Medical Center for Cancer and Cardiovascular Diseases)
Seiya Kato (University of Ryukyus)

O-1 **Triglyceride deposit cardiomyovasculopathy-A novel clinical entity-** ····· 4
Ken-ichi Hirano (Osaka University)

O-2 **ATGL gene mutations: from molecular diagnosis to functional studies** ····· 4
Daniela Tavian (Catholic University of the Sacred Heart, Italy)

O-3 **Neutral lipid storage disease with myopathy in a Chinese family** ····· 5
Yun Yuan (Peking University, P. R. China)

Oral Session 2

Chairpersons: Ken-ichi Hirano (Osaka University)
Robert Zimmermann (Institute of Molecular Biosciences, Graz, Austria)

O-4 **Neutral lipid storage disease with myopathy – pathologically a unique entity among lipid storage myopathies** ····· 5
Ichizo Nishino (National Center of Neurology and Psychiatry)

O-5 **Neutral lipid storage myopathy may respond to beta-adrenergic treatment** ····· 6
Rita Horvath (Newcastle University, UK)

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** ····· 7
Kunihisa Kobayashi (Fukuoka University Chikushi Hospital)

Poster Session 1

Chairperson: Hiroshi Nakamura (Yamaguchi University)

P-1 **Siblings with Lipid Deposition in Cardiomyocytes** ····· 7
Yuji Koide (Nagasaki University)

P-2 **Characteristic feature with infiltration of less intimal macrophage foam cells of arteriosclerotic lesions in triglyceride deposit cardiomyovasculopathy (TGCV)** ····· 8
Yoshihiko Ikeda (National Cerebral and Cardiovascular Center)

P-3 **Raman microscopy analysis of triglycerides deposit cardiomyovasculopathy** ····· 8
Mamoru Hashimoto (Osaka University)

Poster Session 2

Chairperson: Hitoshi Chiba (Hokkaido University)

P-4 **Establishment of the Induction of Vascular Smooth Muscle Cells from the Patient-specific iPS Cells and the fibroblasts** ····· 9
Shunmei Chiba (University of Ryukyus)

P-5 **Biomarker Discovery for Triglyceride Deposit Cardiomyovasculopathy using Proteome and Transcriptome analysis** ····· 9
Naoko Kawasaki (National Institute of Biomedical Innovation)

Poster Session 3

Chairperson: Hironori Nagasaka (Takarazuka City Hospital)

P-6 **Triglyceride Deposit Cardiomyovasculopathy Associated with Type 2 Diabetes Mellitus** ····· 10
Nobuhiro Zaima (Kinki University)

P-7 **Relationship between lipid deposition and ATGL expression in human myocardial tissue: A study with autopsy cases** ····· 10
Seiya Kato (University of Ryukyus)

P-8 **Adipose triglyceride lipase and comparative gene identification-58 are down-regulated in the heart of diabetic fatty db/db mice** ····· 11
Tomoaki Inoue (Kyushu University)

Poster Session 4

Chairperson: Yasuyuki Nagasawa (Osaka University)

P-9 **A Dietary Therapy with Medium Chain Triglyceride for Triglyceride Deposit Cardiomyovasculopathy** ····· 11
Yoko Yasui (Osaka University)

P-10 **Effect of Medium Chain Triglyceride on Adipose Triglyceride Lipase Knockout Mice** ····· 12
Akira Suzuki (Osaka University)

P-11 **New approach for viral myocarditis by MCT diet** ····· 12
Hiroshi Nakamura (Yamaguchi University)

The 6th Meeting of Research Committee for Triglyceride Deposit Cardiomyovasculopathy

Supported by Research Grants for Rare and Intractable Diseases from the Ministry of Health, Labour, and Welfare, the Japanese Government

Organization ····· 13

Sponsorship (in Japanese) ····· 15

O-1 Triglyceride Deposit Cardiomyovasculopathy-A Novel Clinical Entity-

Ken-ichi Hirano
Departments of Cardiovascular Medicine, Osaka University, Japan/The Japan Triglyceride Deposit Cardiomyovasculopathy Study Group

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 for research into heart diseases, but involvement of triglycerides remains unclear. We recently experienced a unique case 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 this phenotype was a novel clinical entity and named it "Triglyceride deposit cardiomyovasculopathy", or simply "Obesity of the heart". The first-identified patient was homozygous for a genetic mutation in the adipose triglyceride lipase (ATGL), an essential molecule for hydrolysis of intracellular triglycerides. Because ATGL deficiency is a rare genetic disorder, I strongly believe that it is essential for researchers and physicians to get together, exchange information and discuss how we diagnose and treat patients in order to overcome this intractable disease as soon as possible. I hope that this symposium will provide such an opportunity.

O-2 ATGL gene mutations: from molecular diagnosis to functional studies

Daniela Tavian
Laboratory of Human Molecular Biology and Genetics, Department of Psychology, Catholic University of the Sacred Heart, Milan, Italy

The lack of adipose triglyceride lipase (ATGL), a patatin-like phospholipase domain-containing enzyme that hydrolyzes fatty acids from triacylglycerols stored in multiple tissues, underlies the autosomal genetic disorder Neutral Lipid Storage Disease with Myopathy (NLSD-M).

In two families of Lebanese and Italian origin presenting NLSD-M, we identified two new missense mutations in highly conserved regions of ATGL (pR221P and pN172K) and one novel nonsense mutation (pW8X). Although the R221P mutation (Lebanese family) did not affect any of the putative catalytic residues or the ability to localize to lipid droplets, it lacked most of lipolytic activity, and cytosolic lipid droplets accumulated in cultured skin patient's fibroblasts. Overexpressing wild-type ATGL in the patient's fibroblasts corrected the metabolic defect and reduced the number and area of lipid droplets. Prediction analysis of the protein structure suggested that the mutation may destabilize a helix proximal to the patatin domain and interfere with enzymatic activity. Despite the poor lipase activity in vitro, the two Lebanese patients, 28 and 33 year old, have only moderate muscle weakness and no apparent myocardial dysfunction. The two Italian patients, 58 and 63 year old, are compound heterozygotes for the other reported mutations. They show a severe myopathy, but only a light cardiomyopathy. Functional studies are in progress in order to verify the pathogenic charge of pN172K mutation, since pW8X can be considered a null protein. The present data show that molecular and functional characterization of ATGL mutations identified in NLSDM patients might provide better insight into disease prognosis.

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

Y Yuan, JJ Chen, DJ Hong, ZX Wang
Department of Neurology, First Hospital of Peking University, P. R. China

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

Ichizo Nishino
Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP)

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

Rita Horvath[2, 3, 12], Peter Reilich[1], Sabine Krause[1], Nicolai Schramm[4], Doug M. Turnbull[2], Michael Trenell[2], Kieren G. Hollingsworth[5], Grainne S. Gorman[2], Volkmar H. Hans[6], Jens Reimann[7], Andrée MacMillan[8], Lesley Turner[8, 9], Annette Schollen[10], Gregor Witte[11], Birgit Czermin[12], Elke Holinski-Feder[12], Maggie C. Walter[1], Benedikt Schoser[1], Hanns Lochmüller[3]

[1]Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University, Munich, Germany

[2]Mitochondrial Research Group, Institute of Ageing and Health, Newcastle University, Newcastle upon Tyne, UK

[3]Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK

[4]Department of Clinical Radiology, Ludwig-Maximilians-University, Munich, Germany

[5]Newcastle Magnetic Resonance Centre, Newcastle University, Newcastle upon Tyne, UK

[6]Institute of Neuropathology, Evangelisches Krankenhaus Bielefeld, Germany

[7]Department of Neurology, University Hospital Bonn, Germany

[8]Provincial Medical Genetics Program, Memorial University, St John´s, NL, Canada

[9]Faculty of Medicine, Memorial University, St John´s, NL, Canada

[10]Institute of Clinical Chemistry, Ludwig-Maximilians-University, Munich, Germany

[11]Department of Biochemistry, Gene Center, Center for Integrated Protein Sciences and Munich Center for Advanced Photonics, Ludwig-Maximilians University, Munich, Germany

[12]Medical Genetic Centre, Munich, Germany

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

Kunihisa Kobayashi[1], Tomoaki Inoue[2], Toyoshi Inoguchi[3], Ken-ichi Hirano[4], Ryoichi Takayanagi[2]

[1]Department of Endocrinology and Diabetes Mellitus, Fukuoka University Chikushi Hospital
[2]Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University
[3]Innovation Center for Medical Redox Navigation, Kyushu University
[4]Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University

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

Yuji Koide[1], Hiroaki Kawano[1], Masayoshi Takeno[1], Kuniko Abe[2], Nobuo Tsuda[2], Ken-ichi Hirano[3], Koji Maemura[1]

[1]Cardiovascular Medicine, Nagasaki University
[2]Pathology, Nagasaki University
[3]Cardiovascular Medicine, Osaka University

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)

Yoshihiko Ikeda[1], Ken-ichi Hirano[1,2,3], Nobuhiro Zaima[5], Nobuyoshi Imai[1], Taka-aki Matsuyama[1], Mitsutoshi Setou[6], Issei Komuro[3], Yoshiki Sawa[4], Japan TGCV Study Group, Hatsue Ishibashi-Ueda[1]

[1]Departments of Pathology, National Cerebral and Cardiovascular Center
[2]Laboratory of Cardiovascular Disease, Novel, Non-invasive, and Nutritional Therapeutics (CNT)
[3]Departments of Cardiovascular Medicine, Graduate School of Medicine, Osaka University
[4]Cardiovascular Surgery, Graduate School of Medicine, Osaka University
[5]Department of Applied Biological Chemistry, Kinki University
[6]Department of Molecular Anatomy, Hamamatsu University School of Medicine

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

M. Hashimoto[1], N. Matsumura[1], H. Niioka[2], T. Araki[1]

[1]Graduate School of Engineering Science, Osaka University
[2]Institute for Nano Science Design, Osaka University

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

Shunmei Chiba[1], Ken-ichi Hirano[2], Seiya Kato[1]
[1]Department of Pathology and Cell Biology, Graduate School of Medicine, University of Ryukyus
[2]Laboratory of Cardiovascular Disease, Novel, Non-invasive, and Nutritional Therapeutics (CNT), Graduate School of Medicine, Osaka University

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

Naoko Kawasaki[1], Ken-ichi Hirano[2], Yasuhiro Hara[1], Jun Adachi[1], Takashi Shiromizu[1], Takeshi Tomonaga[1]
[1]Laboratory of Proteome Research, National Institute of Biomedical Innovation
[2]Cardiovascular Medicine, Osaka University Graduate School of Medicine

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

Nobuhiro Zaima[1*], Yoshihiko Ikeda[2*], Masayuki Mano[3], Hatsue Ishibashi-Ueda[2], Mitsutoshi Setou[4], Ken-ichi Hirano[5]
[1]Kinki University
[2]National Cerebral and Cardiovascular Research Center
[3]Osaka National Hospital
[4]Hamamatsu University School of Medicine
[5]Osaka University
These authors equally contributed to this work.

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

Takaya Nakanishi[1], Hirofumi Matsumoto[1], Shunmei Chiba[1], Yoshihiko Ikeda[2], Hatsue Ishibashi-Ueda[2], Nobuhiro Zaima[3], Ken-ichi Hirano[4], Seiya Kato[1]
[1]Department of Pathology and Cell Biology, Graduate School of Medicine, University of Ryukyus,
[2]Departments of Pathology, National Cerebral and Cardiovascular Research Center,
[3]Department of Applied Biological Chemistry, Kinki University
[4]Laboratory of Cardiovascular Disease, Novel, Non-invasive, and Nutritional Therapeutics (CNT), Graduate School of Medicine, Osaka University

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

Tomoaki Inoue, Kunihisa Kobayashi, Toyoshi Inoguchi, Ryoichi Takayanagi
Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University

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

Yoko Yasui[1], Ken-ichi Hirano[2], Yoshihiko Ikeda[3], Nobuhiro Zaima[4], Yuko Konishi[1], Issei Komuro[2], Norihide Fukushima[5], Masaru Takenaka[6], Mitsutoshi Setou[4], and Masatsugu Hori[7]
[1]Osaka University Hospital
[2]Departments of Cardiovascular Medicine, Osaka University Graduate School of Medicine
[3]Department of Pathology, National Cardiovascular Center
[4]Department of Molecular Anatomy, Hamamatsu University School of Medicine
[5]Cardiovascular Surgery, Osaka University Graduate School of Medicine
[6]Kobe Women's University
[7]Osaka Medical Center for Cancer and Cardiovascular Diseases

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.