

# Two Long-Term Follow-Up Cases Preventing Atherosclerosis by Hormone Replacement Therapy-CETP Deficiency and Tangier Disease



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## Abstract

We reported two cases being treated with HRT for the past 7~11 years presenting no progression in atherosclerosis. The first case was diagnosed of having CETP deficiency (homozygosity type) and fibromyalgia at the age of 52. The HDL-cholesterol level was around 200 mg/ml, CETP DNA point mutation found in intron 14 variant type (A/A) and exon 15 wild type (A/A) were obtained. HRT relieved myalgia and joint pain as well as JFIQ baseline score 91 was reduced to 36, following a 3 months therapy. The second case was diagnosed as a Tangier disease, presenting 1~2 mg/dL of HDL cholesterol and a very low level of LDL-C at the age of 52. Her ANA with anti-centromere antibody 80 at the first visit rose to 640. Depression and shortness of breath disappeared while HRT was continued, However, HRT was discontinued for four years, which made osteoporosis and Alzheimer's disease more progressive. The patient remained in good health as a result of the E3 2 mg/day and atocopherol 600 mg/day that she has been receiving since 2015. In both cases, HRT served as a good therapy for mid aged women in the prevention of atherosclerosis.

**Keywords:** Tangier disease (TD); Cholesterol ester transfer protein (CETP); High density lipoprotein cholesterol (HDL-C); Estrogen progesterone replacement therapy (EPRT); Estradiol (E2); Estriol (E3); Atherosclerosis

**Abbreviation:** TD: Tangier Disease; CETP: Cholesterol Ester Transfer Protein; HDL-C: High Density Lipoprotein Cholesterol; EPRT: Estrogen Progesterone Replacement Therapy; E2: Estradiol; E3: Estriol; HRT: Hormone Replacement Therapy; CEE: Conjugated Equine Estrogen; FSH: Follicle Stimulating Hormone; CAVI: Cardio Ankle Vascular Index; ABI: Ankle Brachial Pressure Index; FMD: Flow Mediated Dilatation; PCI: Percutaneous Coronary Intervention; IMT: Intima Media Thickness; CT: Computed Tomography; ERT: Estrogen Replacement Therapy; ANA: Anti-Nuclear Antibody; SRL: Special Reference Laboratory

## Introduction

Atherosclerosis progression is common in mid-aged women [1]. Especially, in the case of ovarium resection and/or hysterectomy, menopausal symptoms including palpitation, sweating, insomnia, depression, dizziness, shortness of breath and so on are more prevalent and occur earlier in women who did operation than in those who performed no operation [2]. Tangier disease (TD) [3] is very rare. However, it is very important to know whether TD will become progressive or not in patients with atherosclerosis. HDL-C is extremely low such that atherosclerosis might develop, but low

amount of LDL-C will not induce it. In contrast, CETP depletion [4] occurs with very high level of HDL-C, which is thought to be low activity of ABCA1-mediated cholesterol efflux. Therefore, high amount of HDL-C in case of CETP deficiency is not always protective of atherosclerosis. We have experienced two entirely different types of such diseases and treated with hormone replacement therapy (HRT) for the past ten years and reported the clinical significance of HRT for atherosclerosis. As control, 108 specimens from peri-/post-menopausal women were included to explore the influence of HRT for total cholesterol, LDL-C and HDL-C.

## Methods

### Influence of HRT for Levels of T Cholesterol, LDL-C and HDL-C

In addition to the two cases in particular, lipid analysis were performed at 0, 2, 6 and 12 months respectively, with a total of 108 specimen from 95 menopausal women under receiving, tocopherol N 600 mg/day, ERT, estrogen progesterone replacement therapy (EPRT) cyclic, EPRT continuous, 17 $\beta$ E2 patch (0.72 mg/2days) and 17E2 oral(0.5 mg) as an estrogen source and dydrogesterone (5 mg) as a progestin source were used. Conjugated equine estrogen (CEE) was used exceptionally.

### Consent

Two patients' consent were obtained by documentation and the control patients' consent were obtained orally. This study was approved by the Society of Health Care for Menopause and Ageing in Tokyo.

### Detection of Autoantibodies

Anti-nuclear antibody (ANA) was measured by indirect immunofluorescence using Hep  $\square$  cells as a substrate. Most autoantibodies including anti-U1RNP, anti-Sm, anti-SS-A/Ro, anti-SS-B/La, anti-Jo1 and anti-Scl 70/Topo1 were measured by ELISA using commercial kit [5]. Anti-IP3Rs antibodies were detected by immunoblot, which has been previously described [6].

### Determination of Post-Menopause

Postmenopausal conditions were estimated by simplified menopausal index (SMI) [7], menstrual condition, and sex hormone

levels, including estradiol (E2) (less than 45 pg/mL) and/or follicle stimulating hormone (FSH) (more than 27 mIU/mL).

### Genetic Analysis

Gene analysis of TD was done in Fukuoka University School of Medicine and reported in 2009 [8]. CETP deficiency (Reference No:4SSU0001) was determined in Tokyo by Special Reference Laboratory (SRL) in 2009.

### LDL- Cholesterol Estimation

In 2008~2009, the amount of cholesterol in LDL was not directly tested, therefore, it was estimated using Fried Ewald's formula [9].  
LDL cholesterol = Total cholesterol-HDL C-Triglyceride/5

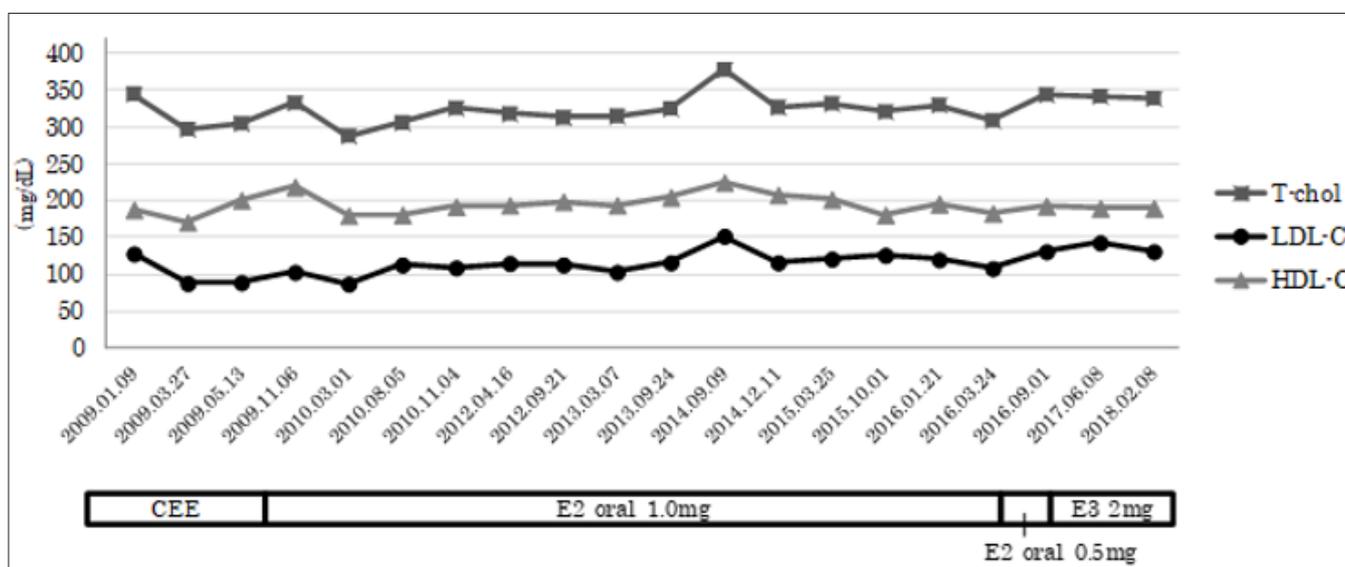
### Atherosclerosis Examination

Atherosclerosis examination included ECG, CAVI (Cardio Ankle Vascular Index), ABI (Ankle Brachial Pressure Index), FMD (Flow Mediated Dilatation) [10], PCI (Percutaneous Coronary Intervention), performing first in early visiting, while the second time was after 8~10 years.

## Results

### Case 1 Female (1955)

- Past history: 29y ovariectomy and hysterectomy,
- Family history: young sister, high HDL- cholesterol
- Present illness: She had complained of knee joint pain for over 30-years, but knee x ray revealed no evidence of abnormality.



**Figure 1:** Long term follow-up of cases with CETP deficiency.

She received CEE (conjugated equine estrogen) 0.625 mg/day from January 2009 to October 2009 and changed oral 17 $\beta$  estradiol (Julina) 1 mg/day from November 2009 to June 2016. Julina was reduced to 0.5 mg/day since July 2018. She has never taken statin or other drugs to control lipids. Total cholesterol, LDL-C and HDL-C level used as baseline 100% in January 2009 were changed to 88%, 69% and 106% in May 2009.

In summer 2009, she complained of bilateral knee, wrist and ankle pains and in September 2009, because of bilateral wrist joint swelling, she could not take a walk with a dog. In January 2011, she visited the clinic owing to fatigue, multiple joint pains and myalgia. She was diagnosed as having fibromyalgia and menopausal arthralgia because of depletion in E2 level (16.0 pg/mL) and FSH level (66.7 mIU/mL). Estrogen replacement therapy reduced Japanese Fibromyalgia Impact Questionnaire (J-FIQ) [11] score from 91 to 36 after two weeks therapy and joint pain reduced by 22% after receiving HRT for 6 months. Laboratory findings and clinical course were presented. For the past 8 years, HDL-cholesterol level was always around 200 mg/mL. LDL-C levels were not so high and maintained the normal range. LDL-C/HDL-C was 0.68 on the first visit. E2 levels after receiving ERT were maintained at 40~90 pg/mL and FSH levels were maintained at 35~70 mIU/mL and the wide spreading pain was reduced to 50%. However, it did not disappear completely (Figure 1). CAVI and ABI revealed 8.9(rt. side)/ 8.6(lt. side) and 1.12(rt. side)/ 1.17(lt. side) in August 2016, suggesting that they were within normal limit. Further FMD showed 11.1% that was normal range (limit level above 6%). Findings from the study of images revealed that carotid artery echogram had no intima media thickness (IMT) hyperplasia and stenosis. Coronary artery computed tomography (CT) scan had no stenosis (in January 2017). Gene analysis was completed in 2011 as shown. One was Intron 14-point mutation (G>A) which causes abnormal splicing (Int 14A mutation). The other was amino acid change (aspartic acid>Glycine) at the position of 442 caused by base change (A>G) at the position of 1506 (D442G mutation)

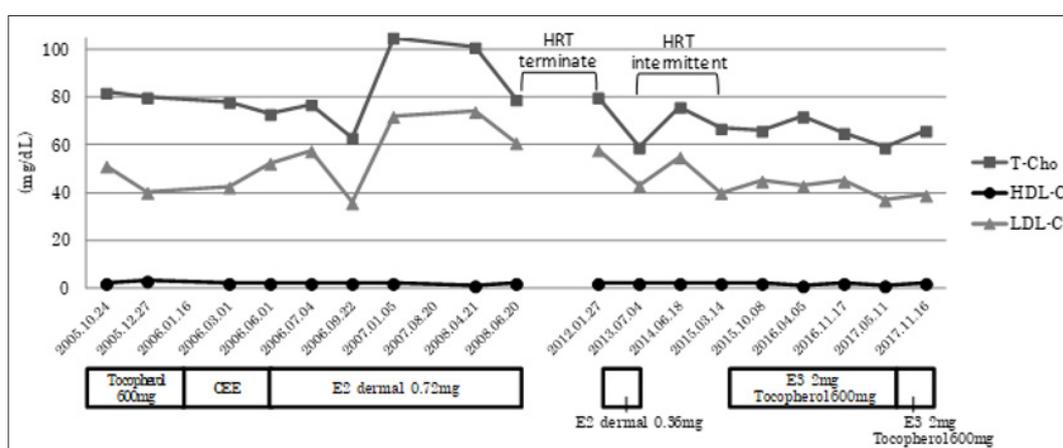
### Case 2, Female, (1953), sales woman

- Chief complaint: lethargy
- Family history: np
- Personal history: np

d) Present illness: In 1990, She was initially told that her serum cholesterol level was extremely low during the annual health check. In 1996, She was found to have uterine myoma and ovarian cyst. Hysterectomy and ovarian cysts resections were done. At that time, she noticed dry mouth. In 2004, Hemorrhoid operation was performed and her platelet count was found to be low. In Jan. 2005, she noticed Raynaud's phenomenon at the both fingers. In July 2005, easy fatigability and general fatigue were experienced. In Oct. 2005, she visited the Keigu Clinic.

### Laboratory Findings and Clinical Course Were Presented

During her first visit in October 2005, T. cholesterol 82 mg/dL, HDL-C 2 mg/dl, triglyceride 144 mg/dL, and estimated LDL-C 52 mg/dl were obtained. LDL-C/HDL-C was 25.5. ANA 40 with discrete speckle and platelet  $8.9 \times 10^4 / \mu\text{l}$  were shown. As sex hormone such as E2 9.0 pg/mL and FSH 42.3mIU/mL was presented at postmenopausal stage, estrogen replacement therapy (ERT) began. Menopausal symptoms including depression, general fatigue and shortness of breath gradually improved when she started taking low dose of ERT in June 2008. She was referred to another hospital because of cognitive disorder and she remained there until January 2012. As soon as she started noticing depression and general fatigue, she returned to receive E3 2 mg/day and tocopherol 600 N mg/day (Figure 2). Moreover, she received bilateral total hip replacement (THR) in February and August 2017 because of walking difficulty owing to osteoporosis. She is now active, but Alzheimer's disease is progressing. CAVI and ABI revealed 8.2(both sides) and 1.07(rt. side)/ 1.09(lt. side) in August 2016, suggesting that they were within normal limit. Further FMD showed 7.9% that fell within normal range. From the study of images, it was revealed that carotid artery echogram had no IMT hyperplasia and stenosis, but coronary artery CT scan presented significant stenosis in the right coronary artery proximal site (in January 2017). Gene analysis was completed in 2011 and shown in previous study [8].



**Figure 2:** Long term follow-up of cases with Tangier disease.

She received CEE from October 2005 to April 2006 and changed  $17\beta$ estradiol patch (estrana) 0.72 mg/2days from June 2006 to July 2008. Since then, HRT was completely terminated for 4 years. She restarted 0.36 mg/2days from January 2012 to July 2013, however, she visited intermittently for two years and has received E3 2 mg/day and  $\alpha$ -tocopherol 600 mg since March 2015. Total cholesterol, LDL-C and HDL.C level as baseline 100% in October 2005 were changed to 97.5%, 76% and 150% in December 2005.

## Discussion

Menopausal women were popularly known to have abnormal lipid and atherosclerosis. In recent times, HRT at onset of menopause is very useful for the prevention of disease progression such as atherosclerosis and osteoporosis [12]. Regarding association with estrogen and lipid, sufficient estradiol (E2) at child bearing stage maintains normal level of total (T)- cholesterol and LDL-C, in contrast to higher levels in menopausal women. Several reasons were considered. The first reason was that, E2 can regulate the function of 3-hydroxy-3 methylglutaryl coenzyme A reductase, consequently maintaining T-cholesterol level [13]. The second reason is that, E2 decreases the function of hepatic lipase and level of LDL-C, resulting from the interfering conversion of IDL -C to LDL-C [14]. The third reason is that, E2 upgrades LDL-receptor on the surface of the hepatic cell and helps the influx of LDL-C to hepatic cell [15]. The fourth, E2 decreases the function of lipo-protein lipase (LPL) to divide triglyceride into free fatty acid and glycerol, and consequently to low level of LDL-C [16].

The fifth, estrogen enhances the synthesis of Apo-A1 as a major part of HDL-C [17], leading to higher HDL-C. Finally, 17 $\beta$ E2 activates CETP, consequently, transporting cholesterol ester in HDL-C to LDL-C, in order to prevent atherosclerosis [18]. In this study, we present two different types of lipidosis. One is TD and the other is CETP deficiency. The HDL-C level ranges from 2 to 200 mg/dL and the LDL-C level ranges from 40 to 140 mg/dL in CETP deficiency. When we saw these patients for the first time, we wondered whether they could remain for a very long time in good health under these conditions of lipid unbalance. Especially, in the second case of Tangier disease, LH ratio was 25.5, indicating the highly progressive condition of atherosclerosis. Almost ten years have passed since we met them, so we decided to assess their health condition especially atherosclerosis under ERT to oral E3 [19] and  $\alpha$ -tocopherol N (600 mg/day)/day. Clinically, it was observed that shortness of breath diminished in both patients undertaking ERT. Probably, micro-coronary artery will be well circulated in such conditions [20].

ECG showed a slight abnormality in ST wave depression, but there is no difference between that of 2009 and 2016. In addition, more test such as cardiac echogram, cervical neck echogram, CAVI, and ABI are not significantly changed. We think ERT is useful for the prevention of atherosclerosis in this case. As for Tangier disease (TD), she had symptomatic Sjogren's syndrome without anti-SS-A/Ro, but high titer of ANA with centromere type. In addition, anti-IP3R antibody such as anti-IP3R1 -146.7 (normal <75.6), anti-IP3R2 -60.8(normal< 48.0) and anti-IP3R3-106.6 (normal< 51.0) were all positive [8]. This result propelled us to develop atherosclerosis because of circulating anti-IP3R antibodies reacting with endothelial cells of vessel. However, she has had no history of severe atherosclerosis, under this condition, because HRT might prevent disease progression. Regretfully, HRT was terminated between 2008 and 2012, owing to movement to the nearest doctor. Unfortunately, osteoporosis and depression became so aggravated that HRT was restarted. At present, she is in good health except for walking with cane. Our case including TD presented not only

extremely low level of HDL-C, but also low level of LDL-C. The latter LDL-C is so low, suggesting that atherosclerosis is not so progressive. She was over 60 years old, but estriol (E3) 2 mg and  $\alpha$ -tocopherol N 600 mg/day were needed for women's health [21] and aging. Unfortunately, we had no chance to detect anti-IP3R Ab in the first case. Recently, extensive atherosclerosis and bleeding tendency in male Tangier disease was reported, accompanied by Leriche syndrome and uncontrollable bleeding [22].

Finally, we evaluated HRT (ERT 10, EPRT 41) or Tocopherol N (600 mg) (57) for normalization of lipid unbalance during the observation period of 2 months only. Although, resulting from small number, 10% reduction in LDL-C level by treatment of tocopherol N 600 mg/day, ERT and EPRT resulted in 7.0%, 30.0% and 19.5%, respectively. And 5 % escalation of HDL-C by them resulted in 35.1%, 30.0% and 29.3%, respectively (Table1). However, in this study, T chol, LDL-C and HDL-C level did not change before and after treatment with tocopherol N 600 mg, ERT and EPRT cyclic or continuous respectively because 17 $\beta$ E2 patch or oral were used in this study instead of CEE [23]. As a limitation,  $\alpha$ -tocopherol N 600 mg did influence 50% of elevation of HDL-C (>3 mg) in TD during an observation period of 2 months. However, if more sensitive detection of HDL-C (Apo A1) would be available, precise assessment would be confirmed. Finally, it was reported that HDL-C increased by 138%, and LDL-C decreased by 40%, following 100 mg of anacetrapib [24]. Although, not available in Japan, we would like to know the efficacy of similar drug for hereditary low HDL-C disease.

**Table 1:** Percent obtaining over 10% reduction in T-cholesterol, LDL-C levels and 5% escalation of HDL-C at 2 months versus baseline under treatment of tocopherol N 600mg/day, ERT and EPRT cyclic/ continuous.

	Case No	T-Chol 10%< down	LDL-C 10%< down	HDL-C 5%< up
TF alone	57	7.0%	17.5%	35.1%
ERT alone	10	30.0%	40.0%	30.0%
EPRT cyclic or EPRT cont alon	41	19.5%	26.8%	29.3%

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## Biographical note

Kiyomitsu Miyachi. MD. Chief doctor for this whole study

Minoru Ihara. MD. Cardiologist performing atherosclerosis tests.

Toshitsugu Ishikawa.MD. An expert for atherosclerosis and lipidosis

Belinda Sasse. MD. Rheumatologist following these patients for 6 months in Japan in 2015

Toshikon Igarashi.PhD. Technician conducting statistical work  
Takao Koyama.MD. Advisor for this work regarding HRT

Keijiro Saku.MD. An expert for atherosclerosis and Tangier disease.

Katsuhiko Mikoshiba.MD. Assisted in the detection of anti-IP3R antibody

Akihiro Inazu.MD. Advisor for the study of CETP deficiency

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