(Table 2B). Almost all plasma ceramide species in apoE$^{-/-}$ decreased with age with the exception of C24:2 ceramide (Table 2B).

The total ceramide level in WT liver increased at 15 w at age (Table 2C) and the levels of C16:0, C18:0, C22:0, and C24:1 ceramides were highest at 15 w of age (Table 2C). In apoE$^{-/-}$ liver, C22:0 ceramide level at 7 w of age was significantly higher than that observed in WT, whereas C22:0 ceramide level and total ceramide level at 65 w of age were significantly lower in apoE$^{-/-}$ than those in WT.

DISCUSSION

The total plasma ceramide level in apoE$^{-/-}$ was comparable to that reported for human atherosclerotic subjects,[14] and was significantly higher than the level observed in WT at all ages, indicating that the aorta of apoE$^{-/-}$ was exposed to a higher plasma ceramide level throughout aging. The difference in the plasma ceramide levels between WT and apoE$^{-/-}$ may be attributed to the LDL level in plasma because LDL is the major ceramide carrier.[27,28] The plasma ceramide level correlated with the LDL cholesterol and oxLDL levels in human samples.[14] The plasma ceramide level in apoE$^{-/-}$ decreased during aging. As a similar phenomemon was reported for oxLDL by Itabe and colleagues[29]: the level of oxLDL increased at 20 w of age and decreased toward the basal level at 40 w of age in apoE$^{-/-}$. These authors suggested that oxLDL appeared before the development of atherosclerotic lesions. Moreover, Schissel *et al.*[30] indicated that ceramides, generated by sSMase from the lesional LDL, but not from the plasma LDL, participated in LDL aggregation. Combining these reports with the results from this study, in which aortic ceramide levels increased at 15 w of age in apoE$^{-/-}$, it is suggested that an elevation in ceramide level at the beginning of atherogenesis is involved in the pathogenesis of atherosclerosis.

Although the aortic ceramide level increased significantly in both WT and apoE$^{-/-}$, the distribution pattern of ceramide species in WT and apoE$^{-/-}$ was different. At 15 w of age, when atherogenesis was assumed to initiate in apoE$^{-/-}$,[31,32] the levels of C16:0, C18:0, C22:0, and C24:0 ceramides in the aorta increased significantly, whereas only C16:0 and C24:1 ceramide levels increased at 65 w of age. The aortic levels of C18:0, C22:0, and C24:0 ceramides caused a similar change in aortic SMase activity. On the basis of these results, we suggest that the increase in C18-24 ceramide levels and the elevation of aortic aSMase activity may play a role in the initiation of atherogenesis. In addition, this is the first study showing elevation of aortic ceramide level during aging in apoE$^{-/-}$ as well as in WT.

In contrast to C24 ceramide, C16:0 ceramide, a major ceramide in the aorta of apoE$^{-/-}$ that increased at 15 and 65 w of age, seemed to have a different function. Ceramide chain length affects the physicochemical properties of lipid membranes;[33] thus, C16:0 ceramide easily mixes with cholesterol in contrast to C24 ceramide.[34] Mesicek *et al.*[35] reported that the overexpression of ceramide synthase (CerS) 5 lead to generation of C16:0 ceramide and an increase in apoptosis, whereas overexpression of CerS2 yielded C24 ceramide and provoked pro-survival signals. Furthermore, in the liver of CerS2 null mice, elevated ROS levels are associated with an increase in C16:0 ceramide and a decrease in mitochondria complex IV activity.[36] In this regard, cellular homeostasis appears to be maintained by a balance between C16:0 and C24:0 ceramides. Although the present study showed that C16:0 ceramide behaved in a manner similar to the very long, unsaturated C24:1 ceramide, the relationship between atherogenesis and apoptosis caused by ceramides, particularly C16:0 and C24 ceramides, requires further examination.

Liver ceramide levels were unchanged with aging in either apoE$^{-/-}$ or WT. Similar results were obtained in diabetic rats.[17] These observations suggested that aging did not affect hepatic ceramide metabolism.

In conclusion, this study suggests that elevation of ceramide species such as C16:0 in the aorta as a result of sSMase activity in the plasma contributes to atherogenesis during aging.

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New Aspects of Statin Safety

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Abstract: Statins have been shown to have beneficial effects on preventing myocardial infarction, revascularization, and stroke, and decreasing cardiovascular mortality in numerous clinical trials. Statins are well tolerated and have been extensively studied all over the world. Physicians should not hesitate in prescribing statins to patients not only with hypercholesterolemia, but also those with high risk for atherosclerotic diseases although statins may have adverse effects such as muscle disease, liver dysfunction, and diabetes, because the incidence of these adverse effects is quite low. Physicians often need to treat patients with hypercholesterolemia and hepatic and renal disease or at the extremes of age with statins to prevent cardiovascular disease. Even in such patients, statins can be safely used with appropriate precautions. Statins sometimes need to be used with other lipid-lowering drugs to reach the recommended LDL-C goal and to improve atherogenic lipid profiles. Combination therapy is generally safe as well, if the drugs are prescribed appropriately and carefully.

Keywords: Cardiovascular disease, combination therapy, diabetes, drug interaction, familial hypercholesterolemia, gene polymorphism, hepatotoxicity, muscle toxicity, renal toxicity, statin.

INTRODUCTION

Statins were developed as competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase to inhibit cholesterol synthesis, resulting in upregulation of the low-density lipoprotein (LDL) receptor and reductions in serum LDL cholesterol (LDL-C). Numerous lines of evidence have demonstrated that statins have beneficial effects on preventing myocardial infarction, revascularization, and stroke, and decreasing cardiovascular mortality. Statins also inhibit isoprenoid intermediates of the cholesterol biosynthetic pathway such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which activate Ras and Rho (Fig. **1**). Thus statins have beneficial effects in cardiovascular disease beyond lowering cholesterol. Current guidelines unequivocally advocate statin therapy for high-risk individuals, and encourage lower LDL-C goals that effectively broaden the pool of individuals eligible for statin therapy. The third report of the National Cholesterol Education Program Adult Treatment Panel (NCEP-ATP) identified an LDL-C goal < 100 mg/dl for high-risk patients with a history of cardiovascular disease, diabetes, or 10-year coronary heart disease risk > 20%. [1]. A subsequent 2004 report from NCEP suggested an optimal goal < 70 mg/dl for patients with the highest risk, including those with established cardiovascular disease as well as additional high-risk characteristics such as diabetes and multiple cardiovascular risk factors, especially cigarette smoking [2]. Statins were shown to be beneficial for reducing LDL-C levels and delay the development of coronary artery disease (CAD) in patients with familial hypercholesterolemia (FH) [3]. Generally, statins are well tolerated. Controlled trials and clinical practice have demonstrated that statins are generally safe; in fact, the incidence of clinically significant side effects is quite low. Furthermore, a recent report of prolonged post-trial follow-up of the Heart Protection Study using simvastatin demonstrated that statin therapy produced larger absolute reductions in the incidence of cardiovascular events, suggesting that prompt initiation and long-term continuation of statin therapy are useful for preventing cardiovascular disease [4]. Indeed, statins are currently the best selling class of prescription drugs worldwide. However, adverse effects such as liver dysfunction, myopathy, and rhabdomyolysis are sometimes quite serious. Today, seven statins, that is, simvastatin, pravastatin, lovastatin, fluvastatin, atorvastatin, pitavastatin, and rosuvastatin, are available in most parts of the world (Fig. **2**). As statins have different LDC-C–lowering efficacies and metabolic and adverse profiles, the most appropriate statin should be selected based on each individual clinical context. The aim of this review is to describe the rate and characteristics of various adverse effects of each statin, as well as combination therapy comprised of statins and other lipid-lowering agents, to provide information for their safe use in various clinical states such as hepatic disease and renal failure.

MUSCLE DISEASES

Muscle toxicity, ranging in severity from myalgia to rhabdomyolysis, is among the most discussed adverse effects associated with statins. Myalgia is defined as muscle ache or weakness without elevated creatine kinase (CK). Myopathy is defined as muscle symptoms including pain and weakness accompanied by a CK elevation more than 10 times the upper limit of the normal range. Rhabdomyolysis is a severe

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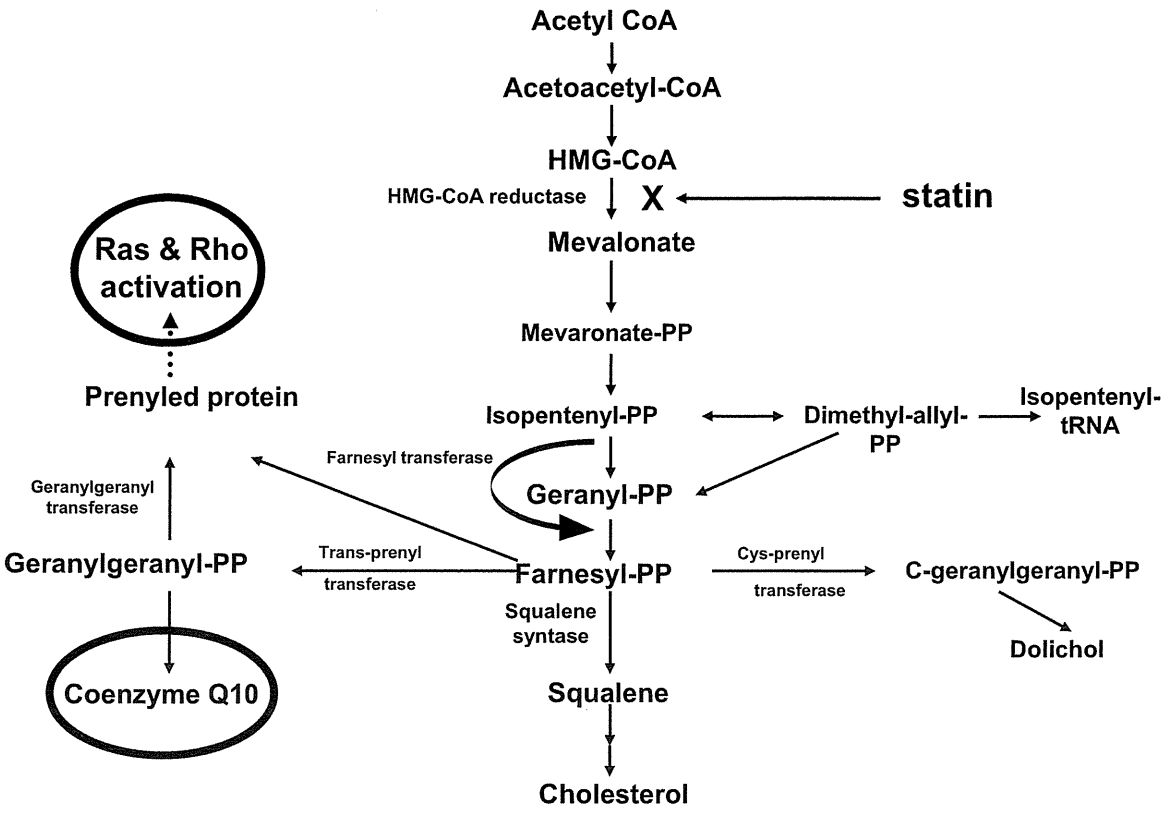

Fig. (1). Cholesterol biosynthetic pathway and site of action of statins. Statins inhibit Ras and Rho activation and Coenzyme Q10 synthesis *via* farnesyl pyrophosphate synthesis.

form of myopathy with symptoms of muscle breakdown and myoglobin release, resulting in brown urine and renal failure. Rhabdomyolysis can be diagnosed by serum CK > 10000 U/L and detection of myoglobin in the urine. Myalgia is sometimes seen with statin use whereas myopathy and rhabdomyolysis are far less common (5.0 and 1.6 per 100000 patient-years, respectively). For example, in the JUPITAR study, only one case of rhabdomyolysis was reported among 8901 patients on rosuvastatin [5]. The incidence of rhabdomyolysis may be different for each statin. A previous report showed that the notification rate of rhabdomyolysis to the Food and Drug Administration (FDA) Adverse Event Reporting System was about four times higher for monotherapy with lovastatin, simvastatin, and atorvastatin (mean rate 0.73; 95% confidence interval (CI), 0.64–0.82 per million prescriptions) than for monotherapy with pravastatin and fluvastatin (mean rate, 0.15; 95% CI, 0.09–0.24 per million prescriptions) [6]. Previous clinical trials have reported that the risk of myopathy is dose-dependent. Uncontrolled trials and post-marketing surveillance suggest that the highest dosage (80 mg/day) of simvastatin causes a higher incidence of rhabdomyolysis than other statins such as lovastatin and atorvastatin. On the other hand, the incidence rate is similar with a lower dosage of simvastatin (< 40 mg/day) [7]. Recently, the FDA has released a warning on the increased risk of muscle injury associated with the 80 mg dose of simvastatin [7].

Several factors may affect the incidence of statin-induced myopathy. Statin therapy should be prescribed with more caution in older patients, especially those more than 80 years, or patients with small body frames and frailty. Statins are not contraindicated in patients with an increased risk for rhabdomyolysis such as renal impairment and hypothyroidism if they have a high risk for cardiovascular disease [8].

The mechanism of muscle injury by statins is unclear. Inhibition of HMG-CoA reductase by statins decreases not only the synthesis of cholesterol but also other products of the mevalonate pathway such as coenzyme Q10 (CoQ10), also known as ubiquinone, which is a central component of the mitochondrial membrane required for oxidative phosphorylation [9-10] (Fig. **1**). Since more than 50% of serum CoQ10 is derived from endogenous synthesis, reductions in serum and muscle CoQ10 are reported in patients treated with statins [11-13]. A previous report suggested that atrogin-1, a key gene involved in skeletal muscle atrophy, may be a critical mediator of muscle injury induced by statins [14]. It has been proposed that statin interaction with the cytochrome P-450 hepatic enzyme system might be related to myopathy. Support for this concept, in part, stems from the enhanced toxicity observed when statins are administered with agents that are also metabolized by the same cytochrome isoforms.

HEPATOTOXICITY AND USE IN PATIENTS WITH HEPATIC DISEASES

Hepatotoxicity induced by statins usually consists of mild increases in hepatic enzymes (AST, ALT, and so on). In the 4S study, there were no significant differences in the elevation of transaminases between the simvastatin and placebo

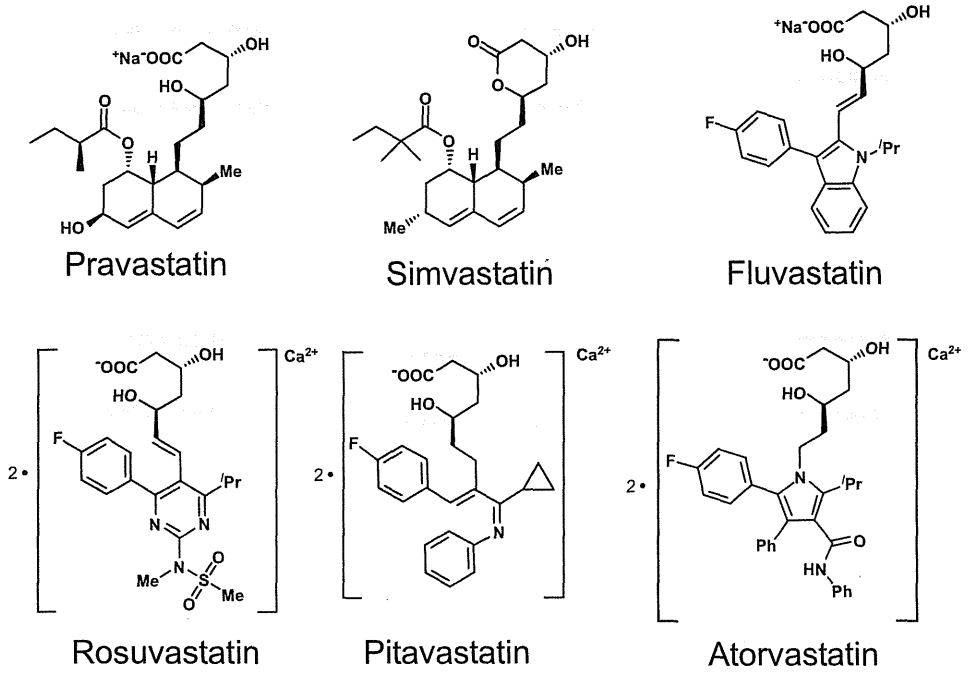

Fig. (2). Chemical structure of each statin. All the statins have carboxyl group that competitively inhibit HMG-CoA.

groups over five years [15]. In the JUPITER study, the incidence of ALT > 3 times the upper normal limit was 0.3% and 0.2% in the rosuvastatin and placebo groups, respectively [5]. According to the FDA Adverse Event Reporting System, 38 cases of liver failure in patients taking statins were reported up to the end of 1999, including eight cases where other causes of liver failure were also present and 30 cases with no other recognized causes [16]. The risk of hepatotoxicity may be increased as the statin dose increases. Meta-analysis from 49 clinical trials involving 14,236 patients revealed that atorvastatin 80 vs. 10 mg was associated with a three-fold increase in the incidence of ALT > 3 times the upper normal limit [17]. Similar findings were observed in the A to Z trial for 80 mg of simvastatin [18]. Thus, the incidence of hepatotoxicity with statin use is extremely low; it is no greater than the risk of liver failure in the general population not taking statins, although high doses of statins may increase the risk of hepatotoxicity.

Previous reports have demonstrated that the prior existence of chronic liver disease does not increase the risk of statin-induced hepatotoxicity. A retrospective cohort study of 93106 patients with established chronic liver disease found that lovastatin exposure was not associated with an increased risk of adverse hepatic outcomes [19]. Tolman *et al.* have demonstrated the relative safety of lovastatin, atorvastatin, and simvastatin versus placebo in patients with nonalcoholic fatty liver disease (NAFLD). Statins have also been reported as safe in cohorts of hepatitis C patients [20]. Recently, one prospective study demonstrated that pravastatin 80 mg in patients with well-compensated chronic liver disease including hepatitis C and NAFLD did not elevate transaminase levels when compared with placebo [21]. Therefore, statins can be safely prescribed to patients with hypercholesterolemia even if they have chronic liver disease.

RENAL INJURY AND USE IN PATIENTS WITH CHRONIC KIDNEY DISEASE

The Renal Expert Panel found no evidence that statins cause acute renal failure or renal insufficiency not associated with rhabdomyolysis. Combined data from three trials of pravastatin showed that renal failure or other renal disease designated as a serious adverse event occurred in 0.5% of the participants on pravastatin and 0.8% of patients on placebo [22]. According to the New Drug Application data, rosuvastatin 80 mg/day was associated with an increased incidence of proteinuria compared to placebo, lower doses of rosuvastatin, and other statins [23]. Prescription data obtained from IMS Health showed the rate of proteinuria with rosuvastatin was similar to simvastatin and pravastatin, but was significantly higher than atorvastatin [24]. In the LIVES study, an increased estimated glomerular filtration rate (eGFR) was observed after 104 weeks treatment with pitavastatin in patients with a baseline eGFR < 60 ml/min/1.73m^2. In a meta-analysis of 13 trials of lipid-lowering drugs in patients with renal disease including 10 statin trials, treated subjects had a lower rate of GFR decline than controls [25]. In patients on dialysis, rosuvastatin 10 mg did not significantly increase the rate of adverse effects compared with placebo [26]. Therefore, statins can be safely used in patients with chronic kidney disease including patients receiving dialysis. On the other hand, diabetic patients with renal impairment should be monitored carefully because of the increased risk of myopathy [27].

RISK OF DIABETES

Recently, a meta-analysis of 13 statin trials with 91,140 patients showed that statin therapy slightly but significantly increased the incidence of diabetes (odds ratio (OR), 1.10; 95% CI, 1.01–1.20) [28]. This study also showed that there

was no clear difference between lipophilic (OR, 1.10; 95% CI, 0.99–1.22) and hydrophilic (OR, 1.08; 95% CI, 0.98-1.20) statins in terms of diabetes risk. Furthermore, another meta-analysis showed that risk of incident diabetes with intensive doses of statins was higher than with moderate doses [29]. However, this report showed that there was one additional case of diabetes for every 498 patients treated for one year, compared with one fewer patient experiencing a cardiovascular event for every 155 patients treated for one year with intensive statin therapy compared with moderate-dose therapy, suggesting that the cardiovascular benefit of intensive therapy may overweigh the risk of diabetes.

Mechanisms to explain the association between statin therapy and incidence of diabetes remain unclear. One possible mechanism is β-cell dysfunction induced by statins. Statins have been shown to inhibit glucose-dependent calcium signaling and insulin secretion [30]. In addition, statins suppress the synthesis of CoQ10, an essential factor in the mitochondrial electron transfer system, resulting in inhibition of insulin secretion due to reduced production of adenosine triphosphate (ATP) [31]. In one meta-analysis, statins had no class effect on insulin sensitivity (standardized mean differences (SMD) −0.084; 95% CI, −0.210–0.042; p = 0.19 vs. control group), although pravastatin improved insulin sensitivity (SMD, 0.342; 95% CI, 0.032–0.621; p = 0.03) whereas simvastatin worsened it (SMD, -0.321; 95% CI, −0.526–−0.117, p = 0.03) [32].

However, the benefits of statin therapy may outweigh the risk of diabetes in patients with moderate or high cardiovascular risk or with existing cardiovascular disease. Indeed, the JUPITER trial indicated that the benefits of rosuvastatin, such as prevention of cardiovascular events and reduction in mortality, exceed the risk of diabetes [33]. Therefore, clinical practice regarding statin therapy does not need to be modified for these patients to avoid the risk of diabetes, although surveillance for glucose tolerance may be necessary.

GENETIC POLYMORPHISMS AND STATIN SAFETY

Recently, genetic polymorphisms have been reported to be associated with an increased risk of statin-induced myopathy. Single nucleotide polymorphisms (SNPs) in the liver uptake transporter OATP1B1 (SLCO1B1) and the efflux transporter BCRP (ABCG2) have been found to alter the disposition and efficacy of statins. Hydrophilic statins such as pravastatin and rosuvastatin are sensitive to SLCO1BI polymorphisms due to their reliance on facilitative transport to reach the pharmacological site of action within hepatocytes. Indeed, patients with SLCOBI polymorphisms had a moderate increase in pravastatin and rosuvastatin plasma exposure (approximately 1.5 to 2-fold) compared with patients with the reference genotype [34]. Similarly, the SLCO1B1 c.521 CC genotype had up to a 3.2-fold increase in simvastatin acid exposure, suggesting that this polymorphism enhanced the therapeutic actions of simvastatin as well as its adverse effect [35]. Indeed, patients with the SLCO1B1 c.521 CC genotype taking 80 mg of simvastatin showed an increased risk of developing myopathy within one year (OR 16.9, compared to the reference genotype) [36]. ABCG2 polymorphisms were reported to affect the metabolism of rosuvastatin. Since BCRP plays a role in both the gastrointestinal absorption and biliary clearance of rosuvastatin, the ABCG2 c.421 > A SNP, caused a 6.9% additional reduction of LDL-C from baseline [37]. Since genetic polymorphisms affecting statins are emerging, we may be able to predict the efficacy and risk of statin therapy on an individual level in the near future.

SAFETY OF STATIN THERAPY IN CHIRDREN WITH FH

FH is an autosomal dominant disorder caused by mutations in genes involved in the LDL receptor pathway. In patients with FH, hypercholesterolemia is observed at birth and premature atherosclerosis begins at a young age. The incidence of myocardial infarction increases at 30 years of age in males and 50 years in females with FH. Though CAD may not be apparent in children with FH, autopsy findings in the Bogalusa Heart Study [38] and the Pathological Determinants of Atherosclerosis in Youth (PDAY) study [39] demonstrated that atherosclerotic changes are already present in children. Therefore, it is advisable to diagnose FH before the age of 10 [40]. The age at which heterozygous FH patients should start drug intervention has not been established. Appropriate LDL-C control should be achieved to prevent premature atherosclerotic changes in the coronary arteries of such patients. The American Academy of Pediatrics proposed that lipid-lowering therapy should be initiated in children with LDL-C of 190 mg/dL or more, those with LDL-C of 160 mg/dL or more with a family history of premature CAD, and those with two or more risk factors. When lifestyle modification is not sufficient, drug therapy should be considered in boys aged 8 to 10 years or older or girls who have reached menarche [41]. In children heterozygous for FH, bile acid resins are the first-line agent, but they do not have high potency for decreasing LDL-C or compliance. Several trials of statin use in children with FH yielded LDL-C reductions of 24.6% to 32.6% [42]. Meta-analysis from six randomized controlled trials of pediatric FH patients aged 8–18 years reported no statistically significant differences between statins and placebo in terms of adverse events (relative risk (RR) 0.99; 95% CI, 0.79–1.25), sexual development (RR of advancing one Tanner stage, 0.96; 95% CI, 0.79–1.17), muscle toxicity (RR of CK 10 times the upper limit of normal (ULN), 1.38; 95% CI, 0.18–10.82), and liver toxicity (RR of three times ULN for AST 0.98; 95% CI, 0.23–4.26; for ALT, 2.03; 95% CI, 0.24–16.95) [43]. This study also found no significant differences in the risk of adverse effects among statins (pravastatin 20–40 mg, simvastatin 40 mg, lovastatin 40 mg, and atorvastatin 10–20 mg) [44]. Therefore, statin therapy in children with FH may be efficacious without untoward effects on safety, although further studies should assess lifelong safety.

SAFETY OF HIGH-DOSE STATIN USE

The third NCEP report states a LDL goal < 100 mg/dl for high-risk patients (existing clinical cardiovascular disease, diabetes, or ten-year CHD risk > 20%) [1]. A subsequent 2004 report from the NCEP suggested an optional LDL goal < 70 mg/dl for those with the highest risk, including patients with established cardiovascular disease plus additional high-risk characteristics such as diabetes mellitus, multiple car-

Table 1. Safety data from randomized controlled trials of intensive statin therapy.

Trials	Stain Comparison	Characteristics of Participation	Alanine Transaminase	Creatine Kinase 10 Times	Rhabdomylosis	Non-vascular Death
	higher vs lower		3 times upper limit of	upper limit of normal, or	higher vs lower	higher vs lower
			normal	myopathy		
			higher vs lower	higher vs lower		
PROVE-IT	A 80 mg vs P 40 mg	Acute coronary syndrome	69(3.3%) vs 23(1.1%)	2(0.1%) vs 3(0.15%)	0(0%) vs 0(0%)	17(0.8%) vs 27(1.3%)
A to Z trial	S 80mg vs S 20 mg	Acute coronary syndrome	19(0.9%) vs 8(0.4%)	9(0.4% vs 1(0.04%)	3(0.1%) vs 0(0%)	21(0.9%) vs 21(0.9%)
TNT	A 80 mg vs A 10 mg	Stable CHD	60(1.2%) vs 9(2%)	(0.0%) vs (0.0%)	2(0.04%) vs 3(0.06%)	158(3.2%) vs 127(2.5%)
IDEAL	A 80 mg vs S 20-40 mg	Stable CHD	43(0.97%) vs 5(0.11%)	6(0.14%) vs 11(0.25%)	2(0.05%) vs 3(0.07%)	143(3.2%) vs 156(3.5%)
SPARCL	A 80 mg vs placebo	Post stroke or TIA	51(2.2%) vs 11(0.5%)	7(0.3%) vs 7(0.3%)	2(0.1%) vs 3(0.1%)	117(4.9%) vs 94(3.9%)

Adapted from Reference 45. A: atorvastatin; S: Simvastatin.

diovascular risk factors including the metabolic syndrome and severe or poorly controlled risk factors, especially cigarette smoking [2]. Therefore, high-risk patients should be treated with high doses of statins. Indeed, in the Treating to New Targets (TNT) trial, based on the standard deviation of LDL at baseline, it can be estimated that approximately 90% of the subjects in the atorvastatin 80 mg group had an LDL-C < 100 mg/dl, whereas approximately 50% of patients had an LDL-C > 100 mg/dl in the atorvastatin 10 mg group [43].

Though high doses of statins were well tolerated in clinical trials of several statins, there was evidence of a higher rate of adverse effects, which led to their discontinuation. In the long-term event trials of atorvastatin 80 mg, the discontinuation rate due to unspecified drug-related adverse events was consistently higher in the patients with high target doses (7% to 10%) than those with moderate target doses (4% to 5%) over the approximately five years of observation. Though no additional risk of myopathy was reported in the higher dose group, elevations in transaminases seemed to be dose-dependent for some statins (Table **1**) [45]. In the A to Z trial, simvastatin 80 mg had a slightly higher rate of treatment discontinuation due to muscle side effects (1.8%) than simvastatin 20 mg (1.5%) [46]. In the SATURN study, a higher incidence of elevated AST levels was observed in the atorvastatin group (2.0% vs. 0.7% with rosuvastatin), and a higher incidence of proteinuria was observed in the rosuvastatin group (3.8% vs. 1.7% with atorvastatin), although both atorvastatin 80 mg and rosuvastatin 40 mg had a low rate of abnormal laboratory values [47]. However, in the pre-approval data trials for 80 mg of rosuvastatin, there was a 1.0% (n =16) incidence of myopathy and seven patients developed rhabdomyolysis, although doses less than or equal to 40 mg had a myopathy rate similar to other statins [45].

To improve patient outcomes, clinical characteristics should be considered in the selection of a particular statin and the dose. Special attention should be given to patients who have renal impairment, hypothyroidism, serious debility, or those who are over 80 years old, especially when a high dose is being considered.

DRUG INTERACTIONS BETWEEN STATINS AND OTHER DRUGS

Statins are rarely associated with significant adverse events at their effective doses. However, when statins are combined with drugs that interact with their metabolism, the risk of adverse events can be significantly increased. The mechanism for most of these drug interactions is associated with the cytochrome P450 system. Drug-specific interactions with each statin are dependent on the metabolic pathway of the statin. Lovastatin, simvastatin, and atorvastatin are metabolized *via* the cytochrome P450 3A4 (CYP3A4) pathway [48]. Drug metabolism studies show simvastatin and lovastatin to be especially sensitive to inhibitory effects on CYP3A4. In previous studies of potent CYP3A4 inhibitors including erythromycin and verapamil, simvastatin levels increased four- to five-fold, and more potent inhibitors such as itraconazole increased simvastatin concentrations by 10- to 20-fold [49-51]. Fluvastatin, which is metabolized by CYP2C9, has been shown to increase the concentration of diclofenac significantly [48]. Both warfarin and phenytoin, which are metabolized by CYP29C, are potentiated when co-administered with fluvastatin. Pravastatin and rosuvastatin are not significantly metabolized by CYP pathways, but gemfibrozil and cyclosporine can increase the concentration of these statins by possibly blocking their biliary excretion [52]. Table **2** shows the profiles of rhabdomyolysis associated with statins reported in the literature [53].

SAFETY OF COMBINATION THERAPY WITH STATINS

Many high-risk patients cannot reach the recommended LDL-C goals with statin therapy alone. Moreover, these patients sometimes have other atherogenic dyslipidemias such as elevated triglycerides (TG), decreased high-density lipo-

Table 2. Profiles of reports of rhabdomyolysis associated with statins.

	Number of	Number of Cases Associated with Potentially Interacting Drugs* (n)	
Statin	Reports/Unique Cases		
Simvastatin	321/215	Mibefradil (48)	Azole antifungals (4)
		Fibrates (33)	Chlorzoxazone (2)
		Cyclosporine (31)	Nefazodone (2)
		Warfarin (12)	Niacin (2)
		Macrolide antibiotics (10)	Tacrolimus (1)
		Digoxin (9)	Fusidic acid (1)
Cerivastatin	231/192	Fibrates (22)	
		Digoxin (7)	
		Warfarin (6)	
		Macrolide antibiotics (2)	
		Cyclosporine (1)	
		Mibefradil (1)	
Atorvastatin	105/73	Mibefradil (45)	
		Fibrates (10)	
		Macrolide antibiotics (13)	
		Warfarin (7)	
		Cyclosporine (5)	
		Digoxin (5)	
		Azole antifungals (2)	
Pravastatin	98/71	Fibrates (6)	
		Macrolide antibiotics (6)	
		Warfarin (5)	
		Cyclosporine (2)	
		Digoxin (2)	
		Mibefradil (1)	
		Niacin (1)	
Lovastatin	51/40	Cyclosporine (12)	Digoxin (2)
		Macrolide antibiotics (11)	Nefazodone (2)
		Azole antifungals (6)	Niacin (1)
		Fibrates (5)	Warfarin (1)
		Mibefradil (3)	
Fluvastatin	11/10	Fibrates (4)	
		Warfarin (2)	
		Digoxin (1)	
		Mibefradil (1)	

*Each case may be associated with one or more potentially increasing drugs.
*There is no data on rosuvastatin in the reference because this report was published prior to the launch of rosuvastatin. Drug surveillance of 8795 cases showed that there were no cases of rhabdomyolysis when rosuvastatin was used in combination with other drugs. However, adverse effects including creatine kinase elevation occurred in combination with fibrates (6/76), macrolide antibiotics (7/37), warfarin (42/359), and antacids (2/17).
Adapted from reference 52.

protein cholesterol (HDL-C). Therefore, other agents are needed to improve these residual lipid abnormalities.

Ezetimibe with Statins

Ezetimibe is a selective cholesterol absorption inhibitor that can reduce serum LDL-C levels. Data from previous randomized clinical trials indicate that the combination of ezetimibe and a statin did not significantly increase the risk of elevated CK, rhabdomyolysis, elevated transaminases, and gastrointestinal adverse events compared to statin monotherapy [54]. Recently, in the SHARP study, compared to placebo, simvastatin plus ezetimibe did not increase the risk of myopathy, hepatitis, and cancer in patients with chronic kidney disease including dialysis patients [55]. Although

data from the SEAS study showed a significant increase in cancer associated with ezetimibe (10.7% with simvastatin plus ezetimibe vs. 7.0% with placebo) [56], analysis of data from the SEAS, SHARP, and IMMPROVE-IT trial did not provide strong evidence of an increased risk of cancer due to ezetimibe (risk ratio of new-onset cancer, 0.96; 95% CI, 0.82–1.12; p = 0.61) [57]. Therefore, long-term use of statin plus ezetimibe combination therapy is considered safe.

Fibrates with Statins

Fibrates exert their effects by activating the peroxisome proliferator–activated receptor α (PPAR-α). Fibrate therapy is consistently associated with substantial decreases in serum TG. Fibrates also increase HDL-C and slightly decrease LDL-C. Given the complementary effects of fibrates and statins on the lipid profile, co-administration of statins and fibrates is potentially an attractive therapeutic option in many mixed dyslipidemia cases. However, the potential risk for myopathy and rhabdomyolysis with combination therapy has been reported. A previous report on drug-specific cohorts of statins and fibrates in the United States showed the incidence of rhabdomyolisis increased to 5.98 (95% CI, 0.72–216.0) per 10,000 person-years for combination therapy of atorvastatin, pravastatin, or simvastatin with a fibrate compared with statin monotherapy [58]. Some fibrates increase the risk of rhabdomyolysis by inhibiting glucuronidation of statins and thereby increasing serum concentrations [59-60]. Indeed, there are large differences in the risk of myopathy between fibrates; there is a greater risk with gemfibrozil than fenofibrate [61]. Gemfibrozil has been found to inhibit statin glucuronidation, whereas fenofibrate has been shown to have no significant effect on statin glucuronidation [62]. Recently, the most important data on the long-term safety of fenofibrate-statin combination therapy has emerged from the ACCORD lipid study [63]. In this study with a mean follow-up of 4.7 years, 5,518 patients with type 2 diabetes treated with simvastatin received either fenofibrate or placebo. There was no significant difference in the incidence of CK elevation between fenofibrate and placebo (0.4% and 0.3%, respectively). An elevation in ALT of more than three times the upper limit of the normal range occurred in 1.9% of the fenofibrate group and 1.5% of the placebo group. However, serum creatinine levels increased more in the fenofibrate group during the course of the trial and, at the last clinic visit, 15.9% of patients in the fenofibrate group and 7.0% in the placebo group were receiving a reduced dose due to decreases in eGFR. Therefore, fenofibrate can be used safely in combination with statins. However, closer monitoring for adverse events in patients with renal impairment, hypothyroidism, or old age is warranted.

Eicosapentaenoic Acid (EPA) with Statins

EPA has beneficial effects in lowering serum TG levels. EPA is generally well tolerated and no drug–drug interactions between EPA and statins are expected. Therefore, EPA–statin combination therapy is an alternative for patients with mixed dyslipidemia. In Japan, long-term efficacy of EPA–statin combination therapy for preventing cardiovascular events was investigated in the JELIS study. This is the only study that evaluated the efficacy of EPA–statin combination therapy. Although gastrointestinal disturbances, skin abnormalities, and bleeding were more common in the EPA group, these adverse effects were mild in nature [64]. More clinical trials are required to define the role of this combination therapy for patients with mixed dyslipidemia.

CONCLUSION

Previous data based from cohort studies and trial databases demonstrate that the rate of adverse effects associated with currently available statins is quite low. Therefore, physicians should not hesitate to prescribe statins to patients with hypercholesterolemia or patients at high risk for CAD to prevent atherosclerosis-related disease. Special attention should be paid, however, when treating patients at high risk of adverse events associated with statins, including liver disease, renal failure, and extremes of age. Furthermore, high-dose statin or combination therapy with other lipid-lowering drugs is sometimes needed to reach the recommended LDL-C goal. To prevent adverse effects of statins, patient characteristics that suggest vulnerability to certain adverse effects, as well as the specific statin and dose, should be thoroughly evaluated.

CONFLICT OF INTEREST

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ABBREVIATIONS

ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
ATP	=	Adenosine triphosphate
CAD	=	Coronary artery disease
CI	=	Confidence interval
CK	=	Creatine kinase
CoQ10	=	Coenzyme Q10
CYP3A4	=	Cytochrome P450 3A4
eGFR	=	Estimated glomerular filtration rate
EPA	=	Eicosapentaenoic acid
FH	=	Familial hypercholesterolemia
HDL-C	=	High-density lipoprotein cholesterol
HMG-CoA	=	3-hydroxy-3-methylglutaryl coenzyme A
LDL-C	=	Low-density lipoprotein cholesterol

NAFLD = Nonalcoholic fatty liver disease

NCEP-ATP = National Cholesterol Education Program Adult Treatment Panel

OR = Odds ratio

PPAR-α = Peroxisome proliferator-activated receptor α.

RR = Relative risk

SMD = Standardized mean differences

SNPs = Single nucleotide polymorphisms

TG = Triglycerides

ULN = Upper limit of normal

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INTERNAL MEDICINE

□ CASE REPORT □

Familial Hypercholesterolemia with Multiple Large Tendinous Xanthomas and Advanced Coronary Artery Atherosclerosis

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Abstract

We herein report the case of a 53-year-old man with severe coronary ischemia who underwent successful coronary artery bypass surgery. Of note, he had hypercholesterolemia and presented with multiple large tendinous xanthomas and thickened Achilles tendons that had been present for more than two decades. Together with a family history of dyslipidemia, the patient was diagnosed as having familial hypercholesterolemia. Irrespective of an extensive search for possible mutations in the genes presumably involved in the patient's pathophysiology, including low-density lipoprotein receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9), autosomal recessive hypercholesterolemia (ARH) and apolipoprotein B (APOB), we were not able to identify the gene mutations responsible for the phenotype observed in the present case.

Key words: familial hypercholesterolemia, tendinous xanthomas, coronary artery disease

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Introduction

Familial hypercholesterolemia (FH) is characterized by increased levels of serum low-density lipoprotein (LDL) cholesterol with a high prevalence of coronary atherosclerosis. It may be inherited as an autosomal dominant trait, and the frequencies of homo- and heterozygotes are estimated to be $1/1\times10^6$ and 1/500, respectively, in the general population. Establishing a diagnosis in these patients is important because lipid-lowering therapy not only slows the progression of atherosclerosis, but also may achieve regression of atherosclerotic vascular lesions. Genetically determining the presence of FH is feasible, although testing is not yet available for routine use. In a large portion of patients, the diagnosis of heterozygous FH is based on laboratory and clinical criteria (1, 2).

Case Report

A 33-year-old man was diagnosed as having hypercholesterolemia more than two decades ago. The lipid profile of his mother at 72 years of age was as follows: total cholesterol, 257 mg/dL; LDL cholesterol, 167 mg/dL; high density lipoprotein (HDL) cholesterol, 72 mg/dL; and triglycerides 138 mg/dL. She was not taking any lipid-lowering drugs nor did she have xanthomas. We could not obtain information regarding abnormal lipid metabolism from other family members. Since then, multiple subcutaneous nodular tumorous lesions began to develop in the present patient and grew larger on the sites of the bilateral Achilles tendons, the ulnar side of the right wrist, the dorsum of the right hand and the

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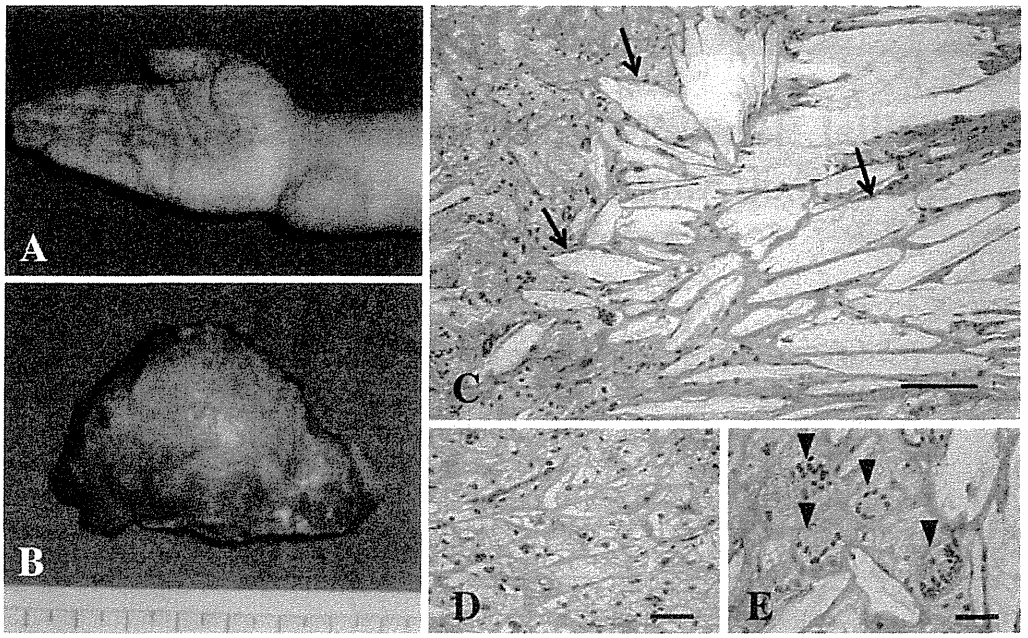

Figure 1. A nodular tumor on the ulnar side of the right wrist observed in 1999 (A). The resected tumor measured 6.0 cm × 3.5 cm in size and was yellowish and lustrous (B). A microscopic examination of the resected tumor revealed numerous extracellular cholesterol deposits (cholesterol clefts) (arrows in C) and diffuse sheets of foamy cells (xanthoma cells) (D) within fibrous tissue. Multinucleated giant cells were also conspicuous (arrowheads in E). The scale bar indicates 100 μm in C (magnification: ×40) and 50 μm in D, E (magnification: ×100).

left fingers. These subcutaneous tumors grew up to the size of a ping-pong ball or an egg. In 1999, at 41 years of age, the patient was admitted to the orthopedics department where some of the subcutaneous tumorous lesions were surgically resected (Fig. 1A, B). A histological examination of the resected specimens revealed numerous extracellular cholesterol (cholesterol clefts) and diffuse sheets of foamy cells (xanthoma cells) interspersed with inflammatory cells within fibrous tissue. Multinucleated giant cells were also conspicuous (Fig. 1C-E). A diagnosis of tendinous xanthomas was then made. The patient's serum LDL cholesterol level was 193 mg/dL at that time, and statin therapy was started thereafter. Meanwhile, however, he did not continue to visit the hospital regularly; therefore, the statin therapy was discontinued 12 years prior to the patient's admission to our hospital in 2010.

In 2007, at 50 years of age, the patient was again diagnosed as having hypercholesterolemia and diabetes mellitus at a routine health check-up; however, medical treatment was not started at that time. In October 2009, he began to experience chest oppression on exertion. The chest pain was associated with cold sweating and subsided when the patient was at rest for five minutes. In May 2010, the duration of chest pain became prolonged up to 20 to 30 minutes and the frequency increased up to three to four times per day. The patient visited a regional hospital where electrocardiogram showed ST-T segment changes suggestive of the presence of unstable angina pectoris. He was immediately hospitalized and an intravenous infusion of heparin sodium and isosorbide dinitrate was started, which markedly, but not completely, relieved his symptoms. He was then transferred to our hospital for further evaluation and treatment.

On admission, the patient's consciousness was clear, his body temperature was 36.8 degrees and his blood pressure was 108/72 mmHg. His heart sounds were normal and no abnormal heart murmurs were audible. No abnormal neurological findings were noted. Chest X-ray and electrocardiogram showed no significant abnormalities on admission. Multiple nodular subcutaneous tumors were observed on the dorsum of the bilateral hands, the bilateral wrists, the soles of the bilateral feet and the bilateral Achilles tendons (Fig. 2A, B). Severe thickening of the bilateral Achilles tendons (right side: 40 mm, left side: 30 mm) was confirmed on an X-ray examination (Fig. 2C, D). Echocardiography demonstrated that the aortic valve had three cusps, while valvular calcification was not significant.

The laboratory data obtained on admission are shown in the Table. At the time of admission, the patient began taking lipid- and glucose-lowering drugs, including rosuvastatin calcium (5.0 mg/d), metformin hydrochloride (750 mg/d), pioglitazone hydrochloride (15 mg/d) and glimepiride (3.0 mg/d). He is a former smoker with a Brinkman index of 390. The serum levels of sitosterol and campesterol measured by means of gas chromatography (SRL, Co., Ltd., Tokyo, Japan) were 1.2 μg/mL and 1.9 μg/mL, respectively, neither of which were elevated.

Coronary angiography showed multivessel coronary artery stenosis with 99% stenosis in the right coronary artery

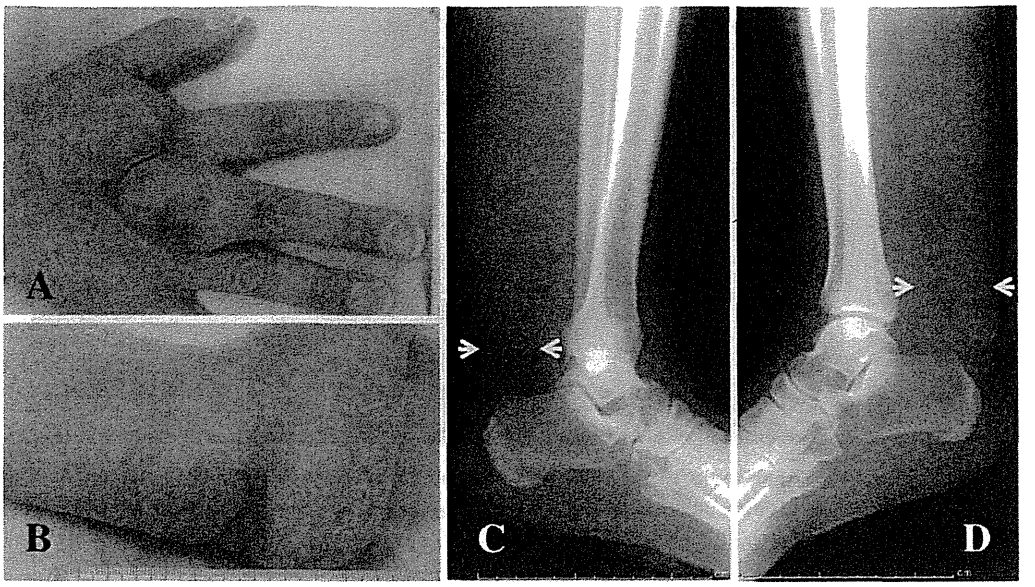

Figure 2. Large nodular tumors on the back side of the right hand (A) and the right Achilles tendon (B) are presented. Radiography of the Achilles tendons showed severe thickening of the bilateral Achilles tendons [left side (arrows in C): 30 mm, right side (arrows in D): 40 mm].

Table. Results of Laboratory Tests on Admission and 1 Month after the Treatment with a Statin and Anti-hyperglycemic Agents

	before	after	normal range
Total Cholesterol	282 mg/dL	137 mg/dL	(130-219)
LDL- Cholesterol	208 mg/dL	71 mg/dL	(70-139)
HDL- Cholesterol	45 mg/dL	41 mg/dL	(40-77)
Triglyceride	143 mg/dL	124 mg/dL	(30-149)
Apolipoprotein B	NE	79 mg/dL	(73-109)
Lipoprotein (a)	NE	23 mg/dL	(< 30)
Glucose	232 mg/dL	96 mg/dL	(60-109)
HbA1c (JDS)	9.3 %	6.2 %	(4.3-5.8)

NE: not examined, JDS: Japan Diabetes Society

(RCA), 90% stenosis in the left anterior descending artery (LAD) and 99% stenosis in the left circumflex artery (LCX) (Fig. 3A, B). No significant luminal stenosis was seen near the ostial lesions of the right or left coronary arteries. The motion of the left ventricle was within normal limits with an ejection fraction of 65.5%. Coronary artery bypass graft surgery was performed on the 14th hospital day. The left internal thoracic artery was anastomosed to the LAD and saphenous vein grafts were anastomosed to the LCX and RCA, which relieved the patient's chest symptoms on exertion.

The results of the activity and gene analysis (BML Co., Ltd., Saitama, Japan) of the LDL receptor (LDLR) were as follows: the LDL-receptor activity in lymphocytes was 137% (normal rage: >80%) and none of the six common LDLR gene mutations of E119K, C317S, 1847T/C, L547V, P664L and K790X were detected.

In addition, DNA sequencing of all of the exons of LDLR and proprotein convertase subtilisin/kexin type 9 (PCSK9) did not show any gene mutations. On the other hand, sequencing of low-density lipoprotein receptor adaptor protein 1 (LDLRAP 1), which is also known as autosomal recessive hypercholesterolemia (ARH), revealed two mutations. A new DNA sequence variation in LDLRAP1 was found, as follows: 604T > C and 654A > G. The frequency of 604T > C was 0.48/0.52 and that of 654A > G was 0.50/0.50 among 31 normal control subjects. The patient had the C/C variation in 604 and the G/G variation in 654. They were both considered to be single nucleotide polymorphisms. We also sequenced the apolipoprotein B (APOB) gene. The G to A mutation at nucleotide 10,708, the major cause of mutations in familial defective APOB, was not detected using asymmetric polymerase chain reaction (PCR) (3) in this patient. The nucleotides 10,564 to 10,884 on Exon 26 of the APOB gene were sequenced in this patient and found to be normal. The sequences of the primers used in this study for sequencing LDLRAP1 (4), LDLR, PCSK9 and APOB are described in the supplementary file.

Discussion

Currently, more than 1,100 mutations occurring at different sites on the LDLR gene in patients with FH have been reported (5). It has been reported that LDLR gene mutations can be identified in 67.8% of FH patients in the Japanese population (6). In the present case, on the other hand, we were unable to identify LDLR gene mutations by screening six commonly observed sites. Patients with these six mutations comprise approximately 30% of the total number of FH heterozygotes investigated in the Japanese population (6). Taking into consideration the finding that the LDLR activity in lymphocytes was within the normal range in this patient, a nonLDLR-mediated mechanism may underlie the pathophysiology in this patient, although the possibil-

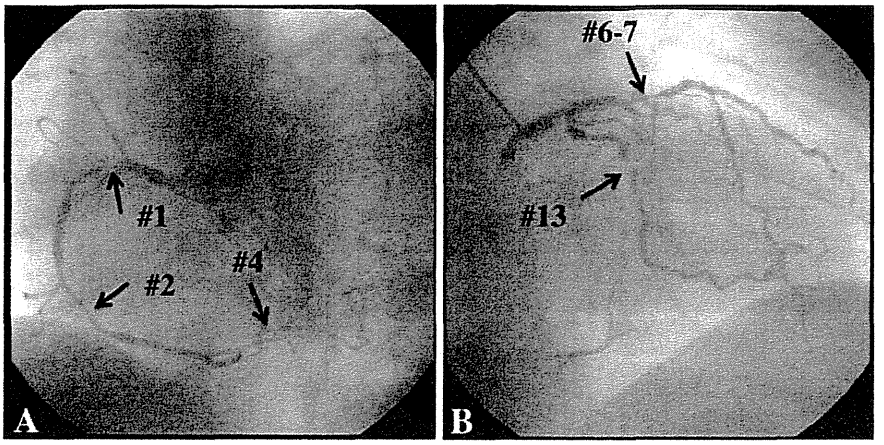

Figure 3. Coronary angiography revealed severe multivessel disease with 99% stenosis at segment (#) 1, 90% stenosis at #2, 99% stenosis at #4 of the right coronary artery (A), 90% stenosis at #6-7 of the left anterior descending artery and 99% stenosis at #13 of the left circumflex artery (B).

ity remains that a mutation of the LDLR gene other than these six mutations may have been present. However, on the other hand, compared with the LDLR gene mutation, fewer studies appear to have investigated the relationship between the LDLR genotype and its activity. A significant minority of patients (approximately 5%) who fulfill the criteria for FH with angiographically-proven coronary disease do not have a defective LDLR function or a detectable mutation in the LDLR gene (7).

Recently, several other genes that are candidates that may explain the FH phenotype have been reported, including APOB, PCSK9 and ARH (8). These nonLDLR mutations are relatively rare compared with the LDLR gene mutations whose prevalence of homozygote and heterozygote in FH are estimated to be $1/1\times10^6$ and 1/500, respectively. Additionally, the homozygote and heterozygote APOB are estimated to be $1/4\times10^6$ and 1/1,000, respectively, the heterozygote of PCSK9 is estimated to be <1/2,500 and the heterozygote of ARH is estimated to be $<1/5\times10^6$. In addition to searching for common LDLR mutations, we performed sequencing of the exons of the LDLR gene, the PCSK9 gene, the ARH gene and the APOB gene; however, no mutations that could explain the overt dyslipidemia observed in our patients were observed.

Xanthomas are a characteristic feature of FH and most usually measure a few centimeters in diameter (9). Multiple large tendinous xanthomas and advanced coronary artery atherosclerosis were noted in the present case. It has been reported that the presence of xanthomas increases the risk of cardiovascular disease in FH patients by as much as three times, suggesting that xanthomas and atherosclerosis may share a certain etiology (10). It has also been suggested that the severity of atherosclerosis and tissue lipid deposition, including the development of xanthomas and the width of the Achilles tendon, is correlated with the duration of hypercholesterolemia or the cholesterol-year score (11). It is not well known whether there is a correlation between the severity of the phenotype of FH, including multiple xanthomas and vascular diseases, and the genotype, including the sites of LDLR gene mutations. In our case, irrespective of the further extensive search for mutations in the LDLR, PCSK9, ARH and APOB genes, we were not able to identify any mutations in these genes that may potentially explain the patient's phenotype. Possible mechanisms, other than the genetic mutations examined in the current study, underlying prominent hypercholesterolemia, as observed in our patient, should be researched and identified in future studies.

The authors state that they have no Conflict of Interest (COI).

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