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The possible role of remnant-like particles as a risk factor for sudden cardiac death

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Abstract Postmortem plasma lipid and lipoprotein levels were analyzed in two groups of Japanese subjects who died suddenly and unexpectedly due to cardiac (n = 93) or non-cardiac (n = 26) causes. No individuals in either group had a significant medical or cardiac history. In this study, we measured plasma total cholesterol, triglycerides, VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol, and especially triglyceride-rich lipoprotein remnants. Triglyceride and apo E-rich remnant-like particles (RLP) were studied as a possible risk factor for sudden cardiac death in relation to the progression of coronary atherosclerosis. The receiver-operating characteristic curve (ROC) analysis showed that RLP-TG was the most significant risk factor for sudden cardiac death among the lipids and lipoproteins and RLP-C was the best predictor for coronary atherosclerosis. HDL-C and LDL-C levels were within normal limits in the majority of the cases and did not appear to relate to the sudden cardiac death. Apo E phenotyping was performed for the detection of the genetic background in the lipid metabolism. The frequency of the Apo E3/3 (wild type) phenotype, which closely relates with the remnant metabolism, was significantly reduced in the sudden cardiac death group. Our study on the postmortem plasma lipid analysis suggested that RLP-C and RLP-TG are the best risk predictor for coronary atherosclerosis and sudden cardiac death, respectively.

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Introduction

Sudden death of cardiovascular origin has been reported to be a major cause of sudden natural death [1]. Advanced atherosclerotic coronary disease is often co-existent, although little or no coronary atherosclerosis was detected in approximately half of the sudden cardiac death cases. In some cases, extremely acute ischemic changes of the myocardium were observed only with microscopically attentive examination, suggesting the possibility of antemortem spasm of coronary stem and disappearance of the coronary thrombus due to the enhanced postmortem fibrinolytic activity [2–4]. Some components in the blood seem to induce the coronary spasm and/or thrombogenesis under certain conditions, which does not necessarily relate with the severity of atherosclerosis.

Risk factors for sudden cardiac death include hypertension, hyperlipidemia, diabetes mellitus, smoking, and chronic stress [5, 6]. The role of various serum lipids and lipoproteins in the pathogenesis of coronary atherosclerosis has been studied extensively during the past decade [7–12]. Increased levels of total cholesterol (TC) and the major cholesterol-carrying lipoprotein, low-density lipoprotein (LDL-C), have been associated with an increased risk of developing coronary artery disease. In contrast, elevated levels of high-density lipoprotein (HDL-C) have a protective effect on the coronary arteries. Hiserodt et al. [13] recently reported that blood lipid and lipoprotein levels in young adults who died from sudden cardiac death were significantly higher than those who died from non-cardiac death, indicating a positive correlation with advanced coronary artery disease. Elevated levels of serum triglycerides (TG) have been suggested as an important independent risk factor for atherosclerosis [14] and coronary artery disease mortality in diabetic patients [15].

Triglyceride-rich, apo E-rich remnant-like particles (RLP), which are almost identical to the remnant lipoproteins found in the blood plasma of type III hyperlipidemic patients [16–19], have been found as atherogenic lipoproteins. Isolation and detection techniques using monoclonal antibodies and ultrasensitive cholesterol and triglyceride methods for RLP have recently been developed by Nakajima et al. [20, 21] and Campos et al. [22]. Using these techniques, the biological activities of remnant lipoproteins have now been elucidated. RLPs-enhanced incorporation of cholesterol into macrophages [23], enhanced platelet aggregation [24], an impaired endothelium-dependent vasorelaxation [25]. These biological characteristics of RLP prompted us to study the role of elevated plasma RLP as a possible risk factor for sudden cardiac death.

We report here a postmortem plasma lipid and lipoprotein analysis on 93 sudden cardiac death and 26 sudden non-cardiac death cases. The purpose of the current study was to explore the relationship among the postmortem plasma levels of RLP-cholesterol (RLP-C) and triglyceride (RLP-TG), the severity of coronary atherosclerosis and sudden cardiac death in order to elucidate the pathogenesis of sudden cardiac death.

Materials and methods

Study population

The study population consisted of a total of 119 individuals aged 20 to 69 years old from the western part of Kanagawa prefecture in Japan who died suddenly and unexpectedly between September 1994 and August 1996. Most of these individuals had no significant history of medical conditions especially cardiac symptoms, and had not taken medication prior to death. An autopsy was performed on all subjects within 12 h after death. The autopsy included pathological examination and biochemical, toxicological, bacteriological and virological testing on body fluids in the Department of Forensic Medicine, Tokai University School of Medicine. The study group (n = 93) consisted of individuals who died from sudden cardiac death and the control group (n = 26) of individuals who died suddenly from non-cardiac causes in the same geographical area during the same period of time. Subjects with ischemic heart failure, i.e. acute myocardial infarction and chronic ischemic heart failure were defined as cardiac death. Congenital anomalies, acquired valvular deformities, idiopathic cardiomyopathy, infective end- or myo-carditis, alcoholic cardiomyopathy [26], fibromuscular dysplasia [27], congestive heart failure i.e. chronic cor pulmonale were excluded. Among the non-cardiac group, most were non-natural deaths such as trauma, accidents and suicides. Cases with fatty liver and renal failure in the control group were excluded because they have already been reported to have higher plasma RLP-C, RLP-TG levels [28–30].

Assessment of severity of coronary atherosclerosis

The grading criteria for severity of coronary atherosclerosis was made following the postmortem protocol of the Department of Forensic Medicine, Tokai University School of Medicine. Briefly, a negative finding (–) indicates no sclerotic changes. The severity was graded if one of the coronary arteries showed fine fatty flecks or streaks (±), atheromatous plaques, i.e. focal thickenings of lipid nature (+), extensive changes of atherosclerosis with or without ulceration (2+), extensive changes of atherosclerosis with calcification (3+), and narrowing of the lumen by more than 75% in at least one portion of a coronary artery (4+).

Lipid and lipoprotein analysis

Blood was removed from the heart and centrifuged to pellet the cells. The supernatant (plasma) was removed and stored at 4° C until assayed for lipids and lipoproteins. Total cholesterol and triglyceride were determined enzymatically by the Fuji Dry Chem cholesterol and triglycerides assay on a Fuji model FDC-5000 automated chemistry analyzer (Fuji Film, Tokyo, Japan). Very low-density lipoprotein (VLDL-C), LDL-C and HDL-C were determined by high pressure liquid chromatography (HPLC) (CCPD, Toso, Tokyo) using methods developed by Hara and Okazaki [31]. RLP-C and RLP-TG were measured by the method of Nakajima et al. [20]. The RLP fraction was separated from the plasma by immunoaffinity mixed gels containing monoclonal anti apo B-100 and anti apo A-I (Japan Immunoresearch Lab., Takasaki, Japan), and the cholesterol and triglyceride levels in this fraction were measured by enzymic cholesterol and triglyceride assays, respectively (Determiner-L TC and Determiner-L TG, Kyowa Medex, Tokyo).

Apo E phenotyping was performed by isoelectric focusing, followed by immunoblotting [32] (Phenotyping Apo E, Joko, Tokyo). Apo E was quantified by a turbidimetry immunoassay (Daiichi Chemical, Tokyo) using polyclonal antiserum raised in goats.

Statistical analysis

The Mann-Whitney U test, a distribution-free non-parametric test, was used to compare the statistical differences between the two groups. Plasma RLP-C and RLP-TG levels have been found to have a non-Gaussian distribution. Diagnostic sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio for a positive test were used to evaluate the clinical performance of the tests. Receiver-operating characteristic (ROC) plots [33] was used to compare the ability of different lipid testing in distinguishing study subjects from control subjects.

Results

Plasma lipid, lipoprotein levels, and the grade of progression of atherosclerosis in coronary arteries were analyzed in 93 individuals who died suddenly and unexpectedly from cardiac causes. Following a complete autopsy, the cause of death in all of these individuals was determined to be sudden cardiac death according to the criteria described in the methods. The control group (n = 26) were analyzed in the same way. The demographic data for the sudden cardiac death and the control group are listed in Table 1.

All subjects in this study were Japanese. The average age in the sudden cardiac death and control groups was 51.3 and 50.3 years, respectively (range 20–69). The frequency of sudden cardiac death was almost 5 times higher in males than in females.

The average weight of the heart in the sudden cardiac death group was 395 g compared to 338 g in the control group ($P < 0.01$). Within the sudden cardiac death group, acute myocardial infarction, chronic ischemic heart disease and acute heart failure of unknown origin were the common findings. The coronary arteries were examined for the grade of atherosclerosis in all sudden cardiac death

Table 1 Demographic data for cases of sudden cardiac death and control

	Cardiac death (n = 93)	Control (n = 26)	P value*
	Mean ± S.D.	Mean ± S.D.	
Age (years)	51.3 ± 14.0	50.3 ± 15.8	NS**
Sex (age)			
Male	79 (51.5 ± 14.2)	22 (48.4 ± 16.0)	NS
Female	14 (50.2 ± 13.2)	4 (60.5 ± 10.2)	NS
Race	Japanese	Japanese	
Heart weight (g)	395 ± 89	338 ± 97	< 0.01
Body weight (kg)	62.7 ± 12.1	56.0 ± 12.1	NS
Body height (cm)	165.1 ± 7.9	161.5 ± 11.8	NS
BMI	22.6 ± 3.6	20.6 ± 3.6	NS
Postmortem time (h)	8.4 ± 3.4	9.0 ± 3.0	NS

* Two-sample Student's t-test
** NS means not significant (≥ 0.05)

and control cases. From the 93 cases of sudden cardiac death, 44 cases showed little or no atherosclerosis in coronary arteries (Table 2), while 49 cases showed advanced coronary atherosclerosis (graded as 2+ and higher). In the control group, fewer cases showed advanced coronary atherosclerosis (9/26). Plasma lipid and lipoprotein levels are shown in Table 3. The median plasma cholesterol and triglyceride levels were significantly higher in the sudden cardiac death group than in the control group (207 mg/dL vs. 135 mg/dL for cholesterol and 421 mg/dL vs. 261 mg/dL for triglyceride). The median levels of RLP-C and RLP-TG were also significantly elevated in the sudden cardiac death group (12.4 mg/dL and 176 mg/dL, respectively) in comparison with the control group (5.4 mg/dL and 63 mg/dL, respectively). VLDL-C, LDL-C and HDL-C were not significantly different between the two groups.

Table 2 Progression of coronary atherosclerosis for cases of sudden cardiac death and control

Cardiac death (n = 93)					
–	±	+	2+	3+	4+
22.6% (21/93)	6.5% (6/93)	18.3% (17/93)	24.7% (23/93)	16.1% (15/93)	11.8% (11/93)
Control (n = 26)					
–	±	+	2+	3+	4+
57.7% (15/26)	7.7% (2/26)	0.0% (0/26)	30.8% (8/26)	3.8% (1/26)	0.0% (0/26)

–: No sclerotic changes (intact)
±: Fine fatty flecks or streaks
+: Atheromatous plaques (focal thickenings of lipid nature)
2+: Atherosclerosis (with or without ulceration in extensive area of coronary artery)
3+: Calcification (atherosclerosis with calcification almost through coronary artery)
4+: Stenosis (narrowing of the lumen more than 75%, at least one portion of coronary artery)

The ROC curve showed that the RLP-TG was the best among different lipid tests for predicting the sudden cardiac death (Fig. 1). The area under the curves for RLP-TG, total triglyceride, RLP-C, total cholesterol, and LDL-C was 0.83, 0.75, 0.72, 0.69 and 0.62, respectively. At its best combination of sensitivity, sepcificity, positive predictive value, negative value and likelihood ratio for a positive test (the point closest to the upper left corner), the RLP-TG level was 85 mg/dL. Using this as the cut-off value for predicting the sudden cardiac death, the sensitivity and specificity was 83% and 85%, respectively. Specifically, 77 out of 93 sudden cardiac death cases had RLP-TG levels higher than 85 mg/dL, whereas only 4 out of 26 control cases had RLP-TG levels higher than this cut-off value. The positive predictive value, negative predictive value and likelihood ratio for a positive test were 95%, 60%, and 5.4, respectively (Table 4).

Table 3 Plasma lipid and lipoprotein levels in cases of sudden cardiac death and control

	Cardiac death (n = 93)			Control (n = 26)			P value*
	Median	25%–75% Tile	Range	Median	25%–75% Tile	Range	
Cholesterol (mg/dL)	207	136 –264	23 – 379	135	67 –197	52 –325	< 0.005
Triglyceride (mg/dL)	421	298 –500	53 –1446	261	169 –353	78 –510	< 0.001
VLDL-C (mg/dL)	16.3	8.4– 32.1	2.4– 116	19.9	9.1– 23.7	5.4–148	NS**
LDL-C (mg/dL)	121	92 –178	52 – 272	116	79 –143	50 –171	NS
HDL-C (mg/dL)	44.6	33.6– 55.9	0.0– 93.9	48.2	21.6– 64.1	0.0–104	NS
RLP-C (mg/dL)	12.4	6.6– 25.2	0.0– 87.9	5.4	3.9– 9.6	0.4– 25.2	< 0.001
RLP-TG (mg/dL)	176	121 –297	2.8– 731	63	29 – 73	19 –286	< 0.001

* Mann-Whitney U test
** NS means not significant (≥ 0.05)

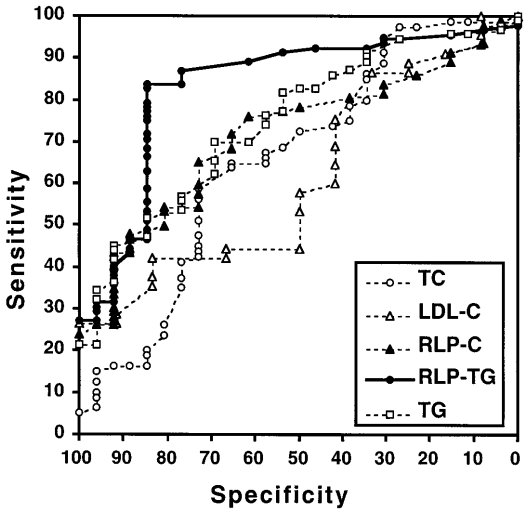

Fig. 1 ROC curves for sudden cardiac death. Lipids and lipoproteins plotted are total cholesterol (TC), LDL-C, RLP-C, RLP-TG and total triglycerides (TG). The sensitivity on the Y-axis is calculated entirely from the sudden cardiac death group and defined as the percentage of cases having lipid and lipoprotein levels higher than a cut-off value [number of sudden cardiac death cases having the levels higher than a cut-off value/total number of sudden cardiac death cases]. The specificity on the Y-axis is calculated entirely from the control group and defined as the percentage of the cases having levels equal to or lower than the same cut-off value

In comparison, RLP-C had the best ability in predicting the presence of coronary atherosclerosis (2+ and higher) (Table 2, Fig. 2). The area under the curve for RLP-C, total cholesterol, RLP-TG, total triglyceride and LDL-C was 0.66, 0.61, 0.43, 0.43 and 0.38, respectively. Estimated from the ROC curve, the best cut-off value for RLP-C to predict the presence of coronary atherosclerosis was 8.0 mg/dL. Using this cut-off value, the sensitivity and specificity was 74% and 58%, respectively. The positive predictive value, negative predictive value and likelihood ratio for a positive test was 63%, 70%, and 1.8, respectively (Table 5).

A genetic factor in lipoprotein metabolism between the sudden cardiac death cases and the controls was studied by apo E phenotyping (Table 6). Plasma apo E levels were not significantly different in the two groups, but the frequency of apo E 3/3 in the cardiac death was reduced to 52.7% compared to 80.8% in the control groups, which was consistent with the average apo E 3/3 frequency detected in Japan [34, 35].

Discussion

We have analyzed postmortem levels of lipids and lipoproteins in 119 individuals (ages 20–69) who died suddenly und unexpectedly due to cardiac or non-cardiac causes. Most of these individuals had no significant history of medical condition, especially cardiac symptoms, and not taken medication prior to death.

Postmortem examination of hearts and coronary arteries from sudden cardiac death individuals revealed advanced coronary atherosclerosis in only half of the sudden cardiac death cases (Table 2). The other half of the cases showed either no or minimal focal atherosclerosis, without narrowing of the lumen. The cases were also examined for the evidence of thrombosis, acute myocardial infarction and chronic ischemic heart diseases. The analysis of other vascular beds including peripheral arteries, renal vessels and cerebral vessels showed only minimal and focal atherosclerosis in the form of minimal focal fatty flecks or streaks.

In order to specify more about the pathogenesis of sudden cardiac death in cases with or without advanced coronary atherosclerosis, we analyzed postmortem plasma samples for the presence of various lipids and lipoproteins, especially RLP. We compared the results to a group of controls analyzed over the same time period who died from non-cardiac-related events. There were clear differences in the plasma levels in lipids and lipoproteins between the two groups, confirming an earlier study by Hiserodt et al. [13].

Total serum cholesterol, lipoproteins and apolipoproteins were stable for at least 24 h after death as already reported by several auhors [36–40]. Särkioja et al. [41] however reported occasional unpredictable fluctuations of lipids and apolipoproteins during the 24 h-period after death and showed that triglycerides were the least stable. The postmorem plasma contained higher percentage of free glycerol (10–60%) measured as triglycerides which may originate from the hydrolysis of triglyceride-rich lipoproteins within the heart. The concentration of free glycerol in the postmortem plasma measured by a different triglyceride reagent was not consistent, indicating the possibility of increased triglyceride hydrolysis by lipoprotein lipase which may be elevated in the postmortem plasma. The RLP fraction may contain free glycerol, which was measured as a part of RLP-TG. A part of free glycerol in

Table 4 Performance of different lipid testing in predicting sudden cardiac death

Lipid Test	Cut-off (mg/dL)	Sensitivity	Specificity	PPV*	NPV**	Likelihood Ratio
RLP-TG	85	83%	85%	95%	60%	5.4
RLP-C	8.0	65%	73%	90%	37%	2.4
Cholesterol	220***	44%	77%	87%	28%	1.9
Triglyceride	150***	96%	12%	80%	43%	1.1
LDL-C	150***	38%	83%	90%	26%	2.3

* PPV means positive predictive value
** NPV means negative predictive value
*** These values are the Japanese upper reference limits in fasting states (Japan Atherosclerosis Society 1987)

the postmortem plasma seemed not bo be deleted by glycerokinase in the TG assay reagent, which can completely delete the free glycerol in plasma from the living subjects [42]. But it still reflected the high total TG level in the blood of cardiac chamber before death.

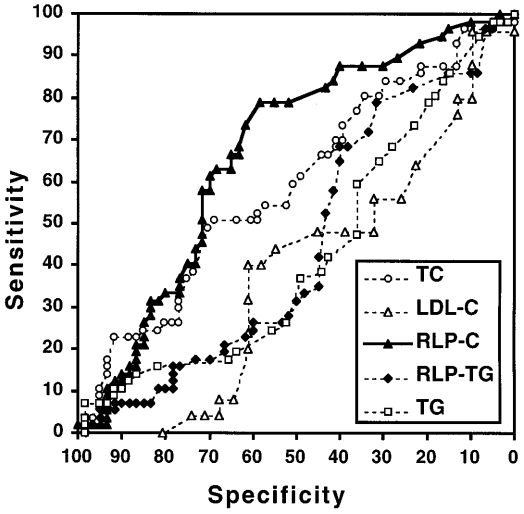

Fig. 2 ROC curves for coronary atherosclerosis. All 119 cases were grouped into those who showed advanced coronary atherosclerosis (2+ and higher in Table 2, $n = 58$, the positive group) and those who did not ($n = 61$, the negative group). The sensitivity is the percentage of the cases having lipid and lipoprotein levels higher than a cut-off value in the positive group. The specificity is the percentage of the cases having levels equal to or lower than the same cut-off value in the negative group

ROC analysis is one of the many ways to compare the clinical performance of different diagnostic tests. Unlike traditionally used diagnostic sensitivity and specificity which use only one cut-off value, ROC plots many, if not all, of the sensitivity and specificity pairs resulting from continuously varying the cut-off values over the entire range of the results obtained. A curve lying above and to the left of another indicates greater clinical performance. In this study, RLP-TG was superior to other lipid testing in predicting sudden cardiac death whereas RLP-C was the best in predicting coronary atherosclerosis (Figs. 1 and 2). By adjusting the cut-off value along the curves, diagnostic sensitivity and specificity can be changed according to desirability to detecting a disease. Decrease in the cut-off value may increase diagnostic sensitivity and sacrifice specificity, and vice versa. In this study, a RLP-TG level of 85 mg/dL is considered to be the best for predicting sudden cardiac death (Table 4). If a cut-off value at 75 mg/dL was selected (the point at the upper right side of the cut-off point of 85 mg/dL in the ROC curve), the diagnostic sensitivity increased from 83% to 87%. But the specificity and likelihood ratio for a positive test decreased from 85% and 5.4 to 77% and 3.8, respectively. A further testing is required to rule out false positive results due to a lower cut-off value.

This is the first time that RLP-TG is shown to be the strongest lipid risk factor for sudden cardiac death. Triglycerides have been suggested to be an important risk factor for coronary heart disease mortality in two epidemiological studies [15, 43]. Results from the Paris

Table 5 Performance of different lipid testing in predicting coronary atherosclerosis

Lipid Test	Cut-off (mg/dL)	Sensitivity	Specificity	PPV*	NPV**	Likelihood Ratio
RLP-C	8.0	74%	58%	63%	70%	1.8
RLP-TG	85	70%	33%	50%	54%	1.1
Cholesterol	220***	49%	71%	61%	60%	1.7
Triglyceride	150***	95%	7%	49%	57%	1.0
LDL-C	150***	24%	61%	44%	39%	0.6

* PPV means positive predictive value
** NPV means negative predictive value
*** These values are the Japanese upper reference limits in fasting states (Japan Atherosclerosis Society 1987)

Table 6 Apo E phenotypes and plasma Apo E levels in cases of sudden cardiac death and control

Cardiac death							
Phenotype	2/2	2/3	3/3	3/4	3/5	Unknown*	ND**
	1.1% (1/93)	3.2% (3/93)	52.7% (49/93)	15.1% (14/93)	5.4% (5/93)	12.9% (12/93)	9.7% (9/93)
ApoE (mg/dL)	8.6	5.8 ± 1.3	4.3 ± 2.4	5.5 ± 2.2	4.3 ± 1.7	3.1 ± 1.5	ND
Control							
Phenotype	2/2	2/3	3/3	3/4	3/5	Unknown	ND
	0.0% (0/26)	0.0% (0/26)	80.8% (21/26)	0.0% (0/26)	0.0% (0/26)	7.7% (2/26)	11.5% (3/26)
ApoE (mg/dL)	–	–	3.6 ± 1.9	–	–	2.70	ND

* Unknown means other phenotypes besides E2, E3, E4
** ND means non-detectable

Prospective Study showed that serum triglyceride level in patients with impaired glucose tolerance who subsequently died of myocardial infarction was significantly higher than that in those who did not die of myocardial infarction [15]. The Apolipoprotein Related Mortality Risk Study (AMORIS) in Sweden demonstrated that there was a 3-fold increase in mortality from fatal myocardial infarction in males with isolated increase in either cholesterol or triglycerides. When both parameters were increased concurrently, there was a 12-fold increase in mortality from myocardial infarction. Increased TG was a strong risk factor for fatal myocardial infarction in females [43]. Our study found that the triglyceride in the RLP fraction (RLP-TG) was superior to the total serum triglyceride and cholesterol levels in predicting sudden cardiac death (Fig. 1).

Elevated levels of plasma RLP-C have been reported as a risk factor for the progression of atherosclerosis [44-48]. Extensive studies have been done regarding the progression of coronary atherosclerosis in type III patients who accumulate remnant lipoproteins in the blood [16–19, 21]. As triglyceride-rich lipoprotein remnants have been defined as catabolites of chylomicrons and VLDL (intermediate lipoprotein), RLP has almost the same characteristics with β-VLDL in type III patients and remains in the plasma with delayed clearance caused by disorders of lipid metabolism. The biological characteristics of RLP have been shown to enhance cholesterol incorporation into macrophages [23], to enhance in vitro platelet aggregation [24] and to impair vasorelaxation [25]. These biological functions of RLP seem to be closely linked with coronary atherosclerosis, thrombosis and spasms [48]. In this study, we found a high correlation between RLP-C levels and the progression of coronary atherosclerosis (Fig. 2). RLP-TG levels were not significantly related to the progression of coronary atherosclerosis.

Apo E phenotyping showed that the frequency of apo E 3/3 (wild type, homozygous of apo ε3 allele) was significantly reduced in the sudden cardiac death group. Apo E 3/3 phenotype is most commonly detected in the population with normal lipid metabolism, with an ε3 allele frequency of 75%–85%, regardless of the race [49]. Apo E 2 or 4 phenotypes have been shown to be more closely associated with remnant metabolism disorder [49, 50]. The increased frequency of the mutant apo E could be the result of reduced frequency of apo E 3/3 phenotypes in sudden cardiac death group. Although unknown phenotypes were detected in 12 cases (12.9%) in the sudden cardiac death group compared to 2 (7.7%) in the control group, it was not clear whether this was the result of other apo E phenotype besides E2, E3 and E4. We confirmed that apo E 3/3 phenotype samples showed ε3 allele in all the cases compared with genotyping by PCR method (data not shown). The apo E 3/3 phenotype seems to be more stable than other phenotypes in the postmortem plasma [51].

In conclusion, we found elevated RLP levels in postmortem plasma which showed the highest correlation with the grade of progression of coronary atherosclerosis and the incidence of cardiac death. Further studies are ongoing to elucidate the mechanism in RLP and the risk for sudden cardiac death. In particular, the relationship with elevated RLP-TG levels and the incidence of sudden cardiac death seems to be worth studying as a risk factor for sudden cardiac death.

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