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ApoB-100 carrying lipoprotein, but not apoB-48, is the major subset of proatherogenic remnant-like lipoprotein particles detected in plasma of sudden cardiac death cases

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Abstract

We have previously reported that plasma levels of remnant-like lipoprotein particles (RLP) significantly increased in sudden cardiac death cases with and without coronary atherosclerosis. In this study we have elucidated the major subset of proatherogenic RLP, containing both apoB-48 and apoB-100-carrying remnants, in plasma of SCD and control death cases.

One hundred and sixty seven Japanese cases of sudden cardiac death and 78 cases of control death underwent autopsy within 12 h after death were studied. Heart weight was 9.2% higher in SCD cases than controls ($P < 0.05$). Moreover 57.5% or 96/167 of the cases had more than grade (2+) coronary atherosclerosis versus 21.8% or 17 of 78 controls ($P < 0.01$). Approximately 2/3 of the cases had full stomach, reflecting the postprandial state at the time of death. Plasma TC, TG, VLDL-C, LDL-C were significantly elevated ($P < 0.001$) together with RLP-C ($P < 0.01$), RLP-TG ($P < 0.005$) in SCD cases. Plasma RLP-apoB-100 levels were significantly elevated in SCD ($P < -0.001$), but apoB-48 levels were not. The median ratio of apoB-100/apoB-48 in RLP was 7.1 in SCD. The median RLP-TG/RLP-C ratio was 4.7, which suggested a large VLDL size. When apoB-48 and apoB-100 in RLP were divided into two groups, above and below the median level, respectively, apoB-48 inversely correlated with RLP-C ($P < 0.05$) and RLP-TG ($P < 0.01$), while apoB-100 in RLP positively correlated with RLP-C ($P < 0.01$) in SCD cases. In conclusion, these results indicated that apoB-100 carrying lipoproteins, not apoB-48 carrying lipoproteins, were the major subset of RLP associated with sudden cardiac death in the postprandial state, regardless to the severity of coronary atherosclerosis.

Keywords: Sudden cardiac death (SCD); Pokkuri death syndrome; Coronary atherosclerosis; Postprandial hyperlipidemia; Remnant-like lipoprotein particles (RLP); ApoB-48; RLP apoB-100; VLDL remnants; Chylomicron (CM) remnants

1. Introduction

Plasma RLP-C levels, as a marker of remnant lipoproteins, are now considered an established risk factor for coronary artery disease (CAD) independent of other lipoproteins [1,2]. Moreover recent evidence suggests that elevated plasma levels of RLP-C and reduced lipoprotein lipase (LPL) activity relate to the promotion of coronary artery events associated with spasm [3–5], which has been often observed as one of the major causes of sudden cardiac death in the Japanese population [6].

We have previously reported the strong association between sudden cardiac death (SCD) and plasma levels of RLP-C and RLP-TG, including cases of Pokkuri death syndrome (sudden cardiac death cases without coronary atherosclerosis observed mostly in Asian populations) [7–12]. SCD cases revealed abnormally high plasma RLP-C and RLP-TG levels in postmortem plasma. RLP isolated from postmortem plasma by immunoaffinity gel separation method [13] induced proatherogenic and proinflammatory

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effects, very similar to RLP isolated from plasma of living subjects [2]. In particular, RLP isolated from plasma of SCD cases was shown to induce severe spasm in vivo in healthy porcine coronary artery [12], which mimicked the etiological phenomenon of Pokkuri death syndrome. Takeichi et al. [9] proposed RLP as one of the major causes of sudden cardiac death and further predicted the presence of a bioactive subset of TG-rich lipoproteins (TRL) in heterogeneous RLP. The heterogeneous composition of RLP in various hyperlipidemia has been reported [13–17] and clarified to contain both chylomicron (CM) (apoB-48 carrying) and VLDL (apoB-100 carrying) remnants in RLP as major subsets of TG-rich lipoproteins.

We have in more than half of SCD observed the cases with presence of a large amount of gastric contents at autopsy, which reflected the postprandial state when sudden cardiac death occurred. We were therefore interested in identifying which remnants, either CM or VLDL remnants, were predominantly associated with the potential risk of coronary artery events in SCD. In order to clarify the characteristics of apoB carrying particles in RLP in SCD cases, we used a recently developed quantitative ELISA assay for determining plasma apoB-48 levels [18], which reflected RLP apoB-48 levels [13]. ApoB-100 levels in RLP were also determined by an assay specific to apoB-100 measurement [20] and the concentrations of apoB-48 and apoB-100 in RLP were compared between SCD and control death cases. In relation to the severity of coronary atherosclerosis and plasma RLP-C, RLP-TG, RLP apB-100 and apoB-48 together with LDL-C, HDL-C or apoB were compared and discussed between SCD and control cases.

The effect of alcohol intake often observed in SCD cases and the kind of foods taken by these cases was also discussed with relation to elevated plasma RLP-C and RLP-TG levels in this manuscript.

2. Materials and methods

2.1. Forensic autopsy cases and blood samples

Autopsy on sudden death cases was conducted at Tokai University School of Medicine, Department of Forensic Medicine from September 1994 to December 2005. They consisted of 202 men and 43 women ranging in age from 20 to 69 years (median 49), from the western part of Kanagawa prefecture in Japan. They had all died suddenly and unexpectedly. Most subjects had no significant history of medical conditions including cardiac symptoms according to their medical records, and had not taken medications prior to death. Autopsy was performed on all subjects within 12 h after death according to the protocol of the Department of Forensic Medicine, Tokai University School of Medicine.

The autopsy included pathological examinations, toxicological, bacteriological and virological tests on body fluids. Individuals with congenital anomalities, acquired valvular deformities, idiopathic cardiomyopathy, infective endomyocarditis, alcoholic cardiomyopathy, fibromuscular dysplasia, congestive heart failure, or cor pulmonale were excluded from the study. Most of the control subjects had died in traffic accidents and from suicide. Individuals with fatty liver and renal failure were also excluded from both groups because of elevated plasma RLP-C and RLP-TG levels [7].

All analysis performed in this study was conducted under the approval of ethical committee of Tokai University School of Medicine.

2.2. Severity assessment of coronary atherosclerosis

The severity of coronary atherosclerosis was graded according to the postmortem protocol of the Department of Forensic Medicine, Tokai University School of Medicine. Briefly, no sclerotic lesion was classified as grade (−), the presence of fatty flecks or streaks in any coronary arteries as (±), the presence of atheromatous plaque such as focal thickening of the lipid nature as (1+), moderate atherosclerotic changes with slight or without ulceration or calcification as (2+), the presence of extensive atherosclerosis with marked ulceration and/or calcification as (3+), and narrowing of the coronary artery as (4+). Scores of (−) and (±) were assigned to cases determined as "without atherosclerosis" as were cases of Pokkuri death syndrome previously reported [9]. The presence of atherosclerosis in Table 1 was the cases above grade (2+), which reflected the clinical feature of coronary atherosclerosis more practically compared with angiographical observation.

2.3. Examination of gastric contents

"Presence" (full stomach) and "absence" (empty stomach) of gastric contents was examined at autopsy in all cases. "Presence" reflected the postprandial state (within 4 h after food intake). "Absence" reflected the fasting state (more than 4 h after food intake).

2.4. Lipid and lipoprotein analysis

Blood was removed from the heart and centrifuged to pellet the cells. The supernatant of the centrifuged blood meant

Table 1
Demographic data of sudden cardiac death (SCD) and control cases

	Control ($n = 78$)	SCD ($n = 167$)	P value
	Mean ± S.D.	Mean ± S.D.	
Age in years	50 ± 13	46 ± 14	NS
Male/female	67/11	135/32	NS
Heart weight (g)	368 ± 84	402 ± 88	<0.05
Body weight (kg)	62.1 ± 10.9	64.2 ± 12.4	NS
Body height (cm)	165 ± 8.5	165 ± 9.2	NS
BMI	22.9 ± 4.3	23.5 ± 3.7	NS
Postmortem time (h)	8.6 ± 3.1	8.4 ± 3.4	NS
Incidence of coronary atherosclerosis above grade (2+)	21.8 (17/78)	57.5 (96/167)	<0.01