

# Small dense LDL-cholesterol is a superior diagnostic marker for familial combined hyperlipidemia

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## INTRODUCTION

Familial combined hyperlipidemia (FCHL) is the most common inherited hyperlipidemia and closely associated with preponderance of coronary heart disease (CHD). FCHL is characterized by several phenotypes, including increased total- and LDL-cholesterol (C), increased triglyceride (TG), increased apolipoprotein B (apoB), and the presence of small dense (sd)-LDL particles. According to the nomogram by the Japanese Atherosclerosis Society, FCHL is diagnosed by the following criteria: 1) the presence of TG  $\geq$  150 mg/dL, and/or LDL-C  $\geq$  140 mg/dL, 2) the high ratio of apolipoprotein (apo) B/LDL-C ( $>$ 1.0), and/or the presence of sd-LDL (mean LDL particle diameter  $<$  25.5 nm), 3) the existence of parents and/or siblings having hyperlipidemia (TG  $\geq$  150 mg/dL and/or LDL-C  $\geq$  140 mg/dL). We established a simple assay for the measurement of sd-LDL-C level directly in serum. The present study was conducted to examine whether sd-LDL-C measurement is a superior diagnostic marker for FCHL to traditional parameters; apoB, LDL size and apoB/LDL-C.

## MATERIALS AND METHODS

**Study Design and Subjects:** 1,527 people were enrolled for this study as normolipidemic and hyperlipidemic (LDL-C  $\geq$  140 mg/dL and/or TG  $\geq$  150 mg/dL) subjects. Informed consent was obtained from all subjects. Subjects taking lipid regulating drugs were excluded from the analysis. Also subjects were excluded from the analysis who were suspected as diabetes mellitus (DM) with their fasting glucose levels ( $\geq$  126 mg/dL) or suspected as familial hypercholesterolemia (FH) with the presence of xanthoma in their Achilles tendons. The analysis was conducted with 1,486 people. FCHL was diagnosed both with the presence of hyperlipidemia (TG  $\geq$  150 mg/dL and/or LDL-C  $\geq$  140 mg/dL) and with the existence of parents and/or siblings having hyperlipidemia and/or being treated with statin or fibrates. Finally, we found 1,205 normolipidemia, 217 hyperlipidemia without no familial history, and 64 FCHL. **Measurement of sd LDL-C and other parameters:** sd-LDL-C was measured by s LDL-C"SEIKEN" using the heparin-magnesium precipitation method (Fig1), and mean LDL diameter was by the 2-16% gradient gel electrophoresis. The diameter smaller than 25.5 nm was diagnosed as sd-LDL phenotype (pattern B). TG and TC were measured by standard laboratory procedures. Apo B was measured by immunotubometric assay (Daiichi Chemical Co. Tokyo, Japan). LDL-C and HDL-C were measured by direct homogenous assays (LDL-EX and HDL-EX, Denka Seiken, Tokyo, Japan).

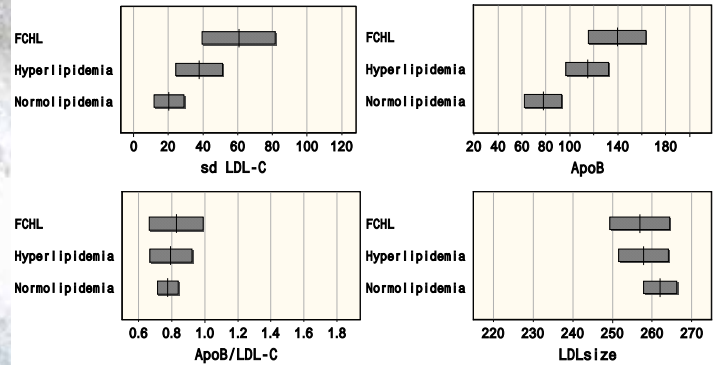
## RESULTS

Table1 shows serum lipid, apolipoproteins, and LDL subspecies in subjects with normolipidemia, hyperlipidemia, and FCHL. sd-LDL-C and apoB were the highest in FCHL and significantly higher in FCHL than in hyperlipidemia ( $p < 0.0001$ ). LDL size was smaller in FCHL than in hyperlipidemia ( $p = 0.244$ ), and the apoB/LDL-C ratio was higher in FCHL than in hyperlipidemia ( $p = 0.002$ ). Fig. 2 shows the ROC curve analysis by sd-LDL-C, apoB, LDL size and the apoB/LDL-C ratio for FCHL diagnosis. The AUCs of ROC curve were 0.822  $\pm$  0.030, 0.798  $\pm$  0.031, 0.625  $\pm$  0.041 and 0.628  $\pm$  0.040, respectively. We determined sd-LDL-C optimal cut-off point as 50 mg/dL, as indicated more than 60% sensitivity and 85% specificity for discriminating FCHL. We compared the sensitivity and specificity for discriminating FCHL from hyperlipidemia among the four parameters: sd-LDL-C ( $\geq$  50 mg/dL), apoB ( $\geq$  120 mg/dL), LDL size (mean diameter  $<$  25.5 nm), and the apoB/LDL-C ratio ( $>$  1.0) (Table2). The specificity was comparable among sd-LDL-C, apoB/LDL-C and LDL size and always over 80% (85.7% by sd-LDL-C). As for the sensitivity, however, there was significant difference between the traditional parameters and sd-LDL-C. While the sensitivity was 67.2% by sd-LDL-C, it was 39.1% by LDL size and 9.4% by the apoB/LDL-C ratio. ApoB showed a good sensitivity (79.7%) but the specificity was very poor (59.8%). Concordance rate was highest by sd-LDL-C (81.5%).

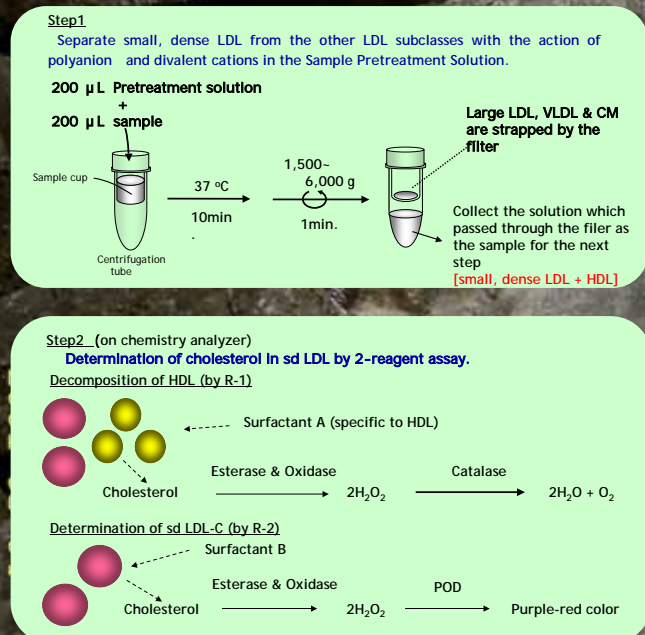
**Table1. Serum lipid and sdLDL-C concentration in subjects with normolipidemia, hyperlipidemia, FCHL.**

	[a] Normolipidemia	[b] Hyperlipidemia	[c] FCHL	[a] vs [b]	[a] vs [c]	[b] vs [c]
n	1,205	217	64			
age (y)	38.7 $\pm$ 12.0	43.6 $\pm$ 10.5	51.7 $\pm$ 12.6	***	***	**
TC (mg/dL)	183 $\pm$ 26	227 $\pm$ 30	285 $\pm$ 41	***	***	***
TG (mg/dL)	73 $\pm$ 30	151 $\pm$ 96	240 $\pm$ 130	***	***	***
HDL-C (mg/dL)	64.6 $\pm$ 15.2	55.6 $\pm$ 13.6	51.9 $\pm$ 12.1	***	***	
LDL-C (mg/dL)	101 $\pm$ 22	147 $\pm$ 27	171 $\pm$ 36	***	***	***
sd LDL-C (mg/dL)	20.2 $\pm$ 8.9	37.7 $\pm$ 13.6	60.6 $\pm$ 21.4	***	***	***
lb LDL-C (mg/dL)	81 $\pm$ 19	109 $\pm$ 26	109 $\pm$ 32	***	***	
ApoB (mg/dL)	78 $\pm$ 16	115 $\pm$ 18	140 $\pm$ 23	***	***	***
LDL size (nm)	26.2 $\pm$ 0.4	25.8 $\pm$ 0.6	25.6 $\pm$ 0.7	***	***	
ApoB/LDL-C	0.786 $\pm$ 0.063	0.794 $\pm$ 0.127	0.843 $\pm$ 0.149	***	***	**

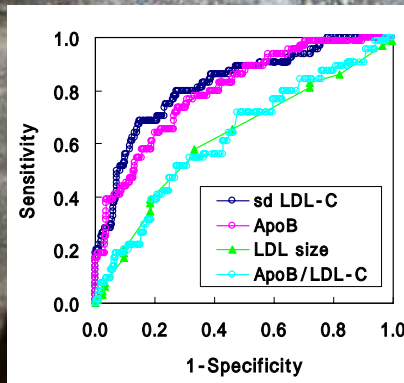
Date are expressed as mean  $\pm$  SD. Normolipidemic = LDL-C $<$ 140mg/dL and TG $<$ 150mg/dL ; Hyperlipidemic = LDL-C $\geq$ 140mg/dL and/or TG $\geq$ 150mg/dL ; FCHL is diagnosed by the presence of hyperlipidemia, and the existence of parents and/or siblings having hyperlipidemia.  
 lb = large buoyant  
 \*p<0.05 , \*\*p<0.01 , \*\*\*p<0.001



**Fig.1. Assay principle for s LDL-C.**



**Fig.2. ROC curves for FCHL diagnosis.**



**Table2. Sensitivity and Specificity for FCHL diagnosis.**

	FCHL		Total	Sensitivity	Specificity	Concordance rate
	+	-				
sd LDL-C	+	43	31	74	85.7%	81.5%
	-	21	186	207	85.7%	
Total	64	217	281			
ApoB	+	51	88	137	79.7%	64.7%
	-	13	129	144	59.4%	
Total	64	217	281			
LDLsize	+	25	40	65	39.1%	71.9%
	-	39	177	216	81.6%	
Total	64	217	281			
ApoB/LDL-C	+	6	9	15	9.4%	76.2%
	-	58	208	266	95.9%	
Total	64	217	281			

## CONCLUSION

These results suggest that quantification of sd-LDL-C determined by the simple precipitation method can be a superior marker for screening of FCHL to other conventional markers.