100 μl of 0.2 mol/l sodium carbonate (pH12). Absorbance was determined at 415 nm with an MPR-4A microplate reader.

2.5. Gene analysis

DNA was prepared from peripheral blood lymphocyte mononuclear cells. Then 1 μg of genomic DNA was subjected to PCR with sense (S) and antisense (AS) primers as follows: 5′-TCTAGGATCACCTCTCAATGGGTCA-3′ and 5′-GGTGGCTTCCACGTGGCTGCCTAAG-3′ [16]. Target DNA sequences were amplified in a 100 μl reaction volume containing DNA template, 100 pmol of the primers, 2.5 mM dNTP, and 1 unit of Ex Taq DNA polymerase followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 63 °C for 0.5 min, and elongation at 72 °C for 0.5 min. One tenth of amplified DNA (285 bp) was amplified in a 8 μl reaction volume with 2 pmol of sense primer, 150 μM dNTP and 3 μM ddNTP mix using the dideoxy-chain termination method of Thermo Sequenase Cy5.5 dye terminator cycle sequencing kit (Amersham pharmacia biotech). Sequence analysis was directly carried out by SEQ4x4 personal sequencing system (Amersham pharmacia biotech) [23].

2.6. Measurement of intima-media thickness (IMT) of the common carotid artery

Examination of IMT was carried out with an ultrasound scanner (SS.D.-1200CV, ALOKA) equipped with a linear 7.5 MHz tranS.D.ucer as previously described [21]. IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall. The measurement of IMT in the common carotid artery was made along a 10 mm-long section just proximal to the carotid bulb [21]. Average IMT was calculated from right and left IMT of common carotid arteries.

2.7. Statistical Analysis

Data are presented as mean ± S.D. The Student's *t*-test was used when means were compared. The counting method and χ^2-test were used to estimate the frequencies of HL genotype. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical characteristics of FCHL

Clinical characteristics and lipid profiles of 49 FCHL and 100 control subjects in this study are shown in Table 1. There is no significant difference between them in male and female, respectively, in age, BMI, TC, LDL-C and TG, as well as, smoking and alcohol consumption (data not shown). The serum levels of TG and apo B in FCHL were significantly higher ($P < 0.01$ and <0.05) than those in control, respectively. The resultant ratio of LDL-C and apo B was significantly lower ($P < 0.01$) in FCHL than that in control. The overall frequencies of C and T allele in the hepatic lipase C-514T polymorphism were 0.35 and 0.65 in male, 0.39 and 0.61 in female, respectively, and the genotype frequencies in both groups were in Hardy–Weinberg equilibrium. The frequencies of genotypes of C/C and T/T in FCHL were significantly lower ($P < 0.05$) and higher ($P < 0.05$) than those in control, respectively, and thus, the overall frequency of T allele in FCHL was significantly higher ($P < 0.05$) than that in control.

3.2. Serum MDA-LDL levels in the patients with FCHL

The circulating MDA-LDL levels were analyzed in FCHL and control subjects using ELISA method with the monoclonal antibody against MDA-LDL, ML25 [4].

Table 1
Clinical and lipid characteristics of male and female FCHL subjects

	Male			Female		
	FCHL	Control	*P*-value	FCHL	Control	*P*-value
Number	31	56		18	44	
Age* (y/o)	56 ± 7	55 ± 5	NS	58 ± 7	58 ± 6	NS
BMI* (kg/m^2)	23.9 ± 0.7	22.9 ± 0.8	NS	23.9 ± 0.9	23.5 ± 0.6	NS
TC* (mg/dl)	241 ± 27	239 ± 20	NS	245 ± 31	244 ± 26	NS
LDL-C* (mg/dl)	149 ± 19	162 ± 15	NS	152 ± 26	159 ± 19	NS
HDL-C* (mg/dl)	44 ± 7	46 ± 7	NS	47 ± 7	49 ± 6	NS
TG* (mg/dl)	234 ± 25	144 ± 16	<0.01	216 ± 27	139 ± 28	<0.01
Apo B* (mg/dl)	145 ± 17	116 ± 21	<0.05	141 ± 19	113 ± 17	<0.05
LDL-C/Apo B*	1.03 ± 0.15	1.40 ± 0.09	<0.01	1.08 ± 0.12	1.41 ± 0.10	<0.01
HL-514 C	0.35	0.48	<0.05	0.39	0.47	<0.05
HL-514 T	0.65	0.52	<0.05	0.61	0.53	<0.05
−514C/C	0.13	0.24	<0.05	0.11	0.23	<0.05
−514C/T	0.45	0.51	NS	0.56	0.48	NS
−514T/T	0.42	0.25	<0.05	0.33	0.29	<0.05

* Data are represented as mean ± S.D.

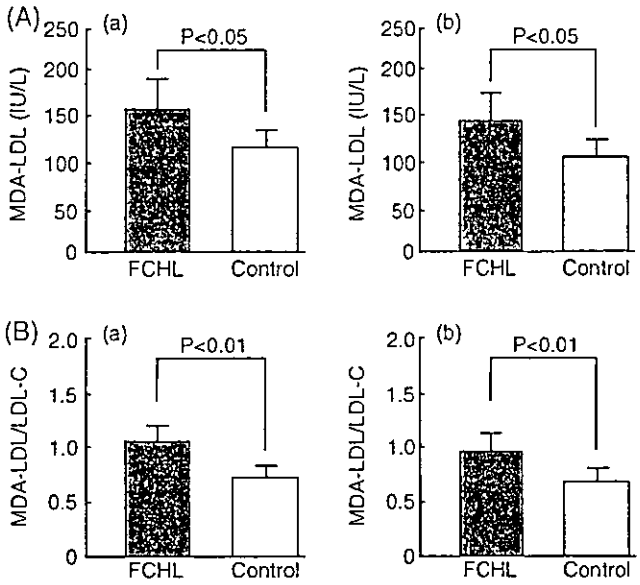

Fig. 1. Comparison of serum MDA-LDL levels between the subjects with FCHL and control. Bar graph shows the serum MDA-LDL levels (A) or the ratios of MDA-LDL and LDL-C (B) of the subjects with FCHL and control in male (a), and female (b), respectively. Data are presented as mean ± S.D.

Serum MDA-LDL levels were 156.4 ± 34.3 (male) and 145.1 ± 29.1 (female) IU/l in FCHL, and 118.1 ± 21.3 IU/l (male) and 109.6 ± 17.0 (female) in control (Fig. 1A panels a and b). There was statistically significant difference of circulating. MDA-LDL levels between FCHL and control both in male ($P < 0.05$) and female ($P < 0.05$). Then, we compared the ratio of MDA-LDL and LDL-C in the both groups. Fig. 1B shows that the ratio of MDA-LDL and LDL-C were 1.04 ± 0.15 (male) and 0.95 ± 0.12 (female) IU/l in FCHL, and 0.74 ± 0.09 IU/l (male) and 0.68 ± 0.09 (female) in control (Fig. 1B panels a and b), showing significantly higher in FCHL compared to control both in male ($P < 0.01$) and female ($P < 0.01$). These results indicate that the circulating MDA-LDL level and the proportion of MDA-LDL in LDL are increased both in the male and female subjects with FCHL.

We have shown that the positive correlation of MDA-LDL with LDL-C and TG, and the negative correlation with

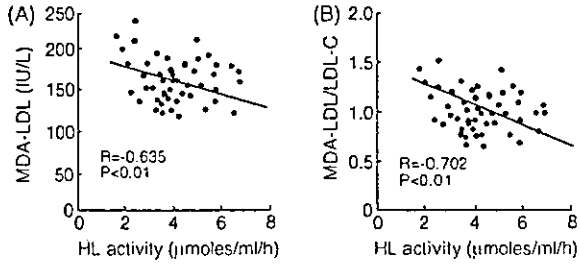

Fig. 2. Correlation of hepatic lipase activity with serum MDA-LDL level (A) or the ratio of MDA-LDL and LDL-C (B).

HDL-C in the serum [12]. The correlation of MDA-LDL with other lipid levels, together with the proposal causative importance of HL in the FCHL subjects [13], prompted us to investigate the effect of HL activity on the circulating MDA-LDL level. Fig. 2A shows the relationship of post-heparin HL activity and serum MDA-LDL level in the FCHL subjects. The HL activity was significantly negatively correlated with MDA-LDL ($R = -0.635$, $P < 0.01$). Furthermore, as shown in panel B, the activity was correlated with the ratio of MDA-LDL and LDL-C ($R = -0.702$, $P < 0.01$). These correlation of MDA-LDL and serum HL activity suggests that the involvement of HL in the determination of circulating MDA-LDL possibly through the TG and HDL-C metabolism in these subjects.

3.3. The C-514T HL polymorphism and serum MDA-LDL level in FCHL

Table 2 shows the serum lipid and apo B levels in the male and female FCHL subjects with each genotype of C-514T HL polymorphism. Serum levels of TC, LDL-C, HDL-C and apo B were not significantly different among the genotypes in the FCHL subjects. However, the serum TG levels in the subjects with T/T or C/T genotype were significantly higher ($P < 0.05$) than that with C/C genotype in male. The ratio of LDL-C and apo B was significantly decreased in the subjects with T/T or C/T genotype compared to that with C/C in male.

Fig. 3 shows the serum MDA-LDL levels and the ratio of MDA-LDL and LDL-C in the FCHL subjects with

Table 2
Serum levels of lipid and apolipoprotein B in male and female FCHL subjects with each HL genotype

	Male			Female		
	C/C	C/T	T/T	C/C	C/T	T/T
TC (mg/dl)	234 ± 29	240 ± 26	249 ± 29	245 ± 36	245 ± 25	246 ± 17
LDL-C (mg/dl)	148 ± 24	148 ± 16	150 ± 23	151 ± 28	151 ± 27	154 ± 18
HDL-C (mg/dl)	45 ± 8	44 ± 6	43 ± 8	49 ± 7	46 ± 6	47 ± 8
TG (mg/dl)	199 ± 33	241 ± 21*	271 ± 32*	219 ± 34	213 ± 21	215 ± 20
Apo B (mg/dl)	139 ± 22	145 ± 16	149 ± 19	139 ± 19	141 ± 18	142 ± 20
LDL-C/Apo B	1.07 ± 0.07	1.03 ± 0.05*	1.02 ± 0.05*	1.09 ± 0.09	1.07 ± 0.05	1.08 ± 0.06

Data are represented as mean ± S.D., *$P < 0.05$ vs. C/C.

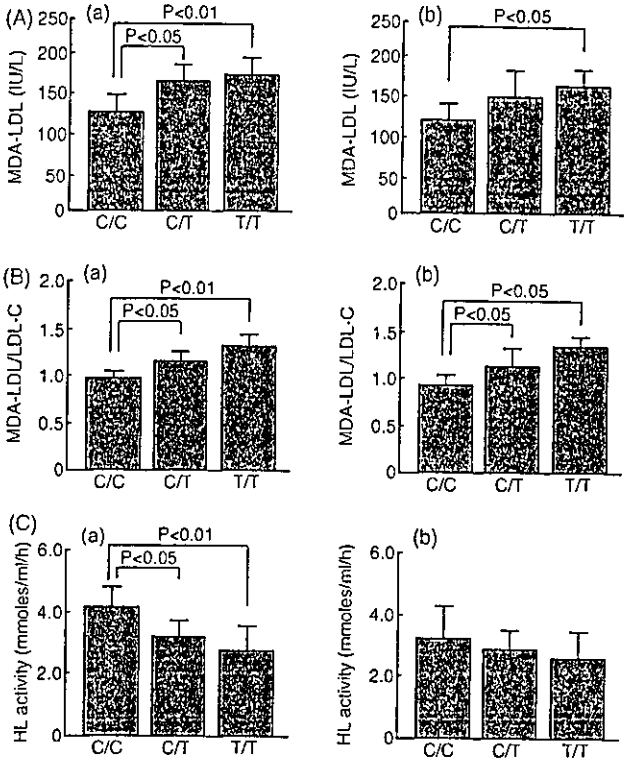

Fig. 3. Comparison of serum MDA-LDL levels (A), the ratio of MDA-LDL and LDL-C (B), and hepatic lipase activities (C) among the FCHL subjects with each genotype of HL C-514T polymorphism in male (a), and female (b), respectively. Data are presented as mean ± S.D.

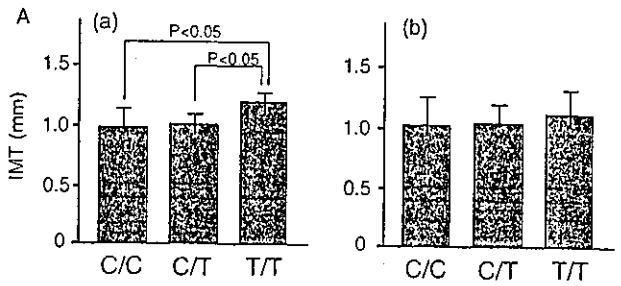

Fig. 4. Comparison of IMT among the FCHL subjects with each genotype of HL C-514T polymorphism in male (a), and female (b), respectively. Data are presented as mean ± S.D.

each genotype. The serum MDA-LDL levels in the male subjects with T/T or C/T genotypes were 173.1 ± 21.0 and 161.2 ± 22.6, respectively, and were both significantly increased ($p < 0.05$ and $P < 0.01$, respectively) compared to that with C/C genotype (128.8±20.6) (Fig. 1A, panel a). The serum MDA-LDL level in female was significantly increased ($p < 0.05$) in the subjects with T/T genotype (156.9±21.5) compared to that with C/C genotype (122.2 ± 17.1). As shown in Fig. 1B, the ratio of MDA-LDL and LDL-C in the male subjects with T/T (1.26 ± 0.11) or C/T (1.13 ± 0.08) genotype was significantly higher ($p < 0.01$ and $p < 0.05$, respectively) than that with C/C genotype (0.99 ± 0.05). The ratio of MDA-LDL and LDL-C in the female subjects with T/T (1.24 ± 0.08) or C/T (1.10 ± 0.13) genotype was also significantly higher (both $p < 0.05$) than that with C/C genotype (0.93±0.09). Fig. 1C shows the HL activity in the subjects. The subjects with T/T and C/T genotypes showed the activities to 65 % ($P < 0.05$) and 77% ($P < 0.01$) of that in the subjects with C/C genotype in male, 79 and 89% of that in female, respectively. These results show that the circulating MDA-LDL level and the proportion of MDA-LDL in LDL are increased in the subjects with T allele both in male and female. The difference of circulating MDA-LDL level is possible to be consistent with the differing HL activity levels in the subjects with each HL C-514T polymorphism (Fig. 4).

3.4. Carotid atherosclerosis and HL C-514T polymorphism

We finally analyzed the IMT of carotid arteries in the FCHL subjects in order to know the clinical significance of the increased circulating MDA-LDL levels in the subjects with each genotype of HL C-514T polymorphism. The values of IMT in the subjects with C/C, C/T and T/T genotypes were 1.00 ± 0.14, 1.03 ± 0.06 and 1.21 ± 0.04 in male, and 1.04 ± 0.19, 1.06 ± 0.10 and 1.11 ± 0.12 in female, respectively. The IMT in male was significantly higher in the subjects with T/T genotype than those with C/T or C/C genotype (both $P < 0.05$). These results suggest that the male FCHL subjects with T/T genotype show the advanced carotid atherosclerosis as well as the increased MDA-LDL levels in the sera.

4. Discussion

This study presented that the circulating MDA-LDL level in the patients with FCHL is increased compared to those without FCHL, which are adjusted about the age, serum TC, LDL-C and HDL-C levels. The MDA-LDL level is negatively correlated with hepatic lipase activity in their sera. Furthermore, the circulating MDA-LDL level was significantly higher in the patients with T allele in the HL C-514 T polymorphism than those with C allele. The patients with T allele showed the advanced carotid intima-media thickness in male. These findings indicate that the circulating MDA-LDL level is possibly contributing the atherosclerosis in FCHL, and the common HL polymorphism might be a determinant of the serum level of oxidazed LDL in the patients with FCHL.

Oxidized LDL, as modified LDL, is thought to play key roles in the progression of atherosclerosis [24–26]. The detection and quantification of circulating oxidized LDL potentially reflect the severity and phases of atherosclerosis. Using a sensitive method to measure the circulating MDA-LDL, we have reported that its level is increased in the patients with CAD [12]. Our and recent other investigations to date revealed that the measurements of serum oxidized LDL is useful for the identification of patients

with acute coronary syndrome [8–12]. On the basis of these accumulating evidence, in this study, in order to know the clinical significance of the circulating level of oxidized LDL as a predictor of atherosclerosis, the serum levels of MDA-LDL in the patients with FCHL, which is known to cause the advanced atherosclerosis, were analyzed. Previous study has shown the positive correlation of serum levels of MDA-LDL with LDL-C and TG, and the negative correlation with HDL-C and averaged particle size of LDL [12]. The close relationship of the circulating MDA-LDL level and TG level, in addition to the LDL-C level seems indicative for the common pathways of the atherogenic oxidized lipoproteins derived from TG-rich lipoproteins. This study showed that the MDA-LDL levels and the ratio of MDA-LDL and LDL-C in the FCHL subjects were negatively correlated with the HL activity in post-heparin plasma, respectively. These findings suggest that the atherogenic property in the concern of MDA-LDL might be caused by the higher production of the modified LDL from the TG-rich lipoproteins accompanied with disturbed HL activity, although the further mechanism should be studied in detail. Recent study by Dugi et al. showed that the low HL activity is a risk factor for CAD using the patients with CAD and familial hypercholesterolemia (FH) [27]. Together with the present analysis, the dyslipidemia associated with low HL activity seems to be associated with atherosclerosis, particularly in the patients with a high risk for CAD, such as FCHL and FH.

This study presented the characterization of lipid profile of FCHL in association with the common promoter polymorphism, C-514T, on the *HL* gene, which has been established as a genetic marker affecting on the enzyme activity and plasma lipid levels in general populations by previous studies [13]. The lipid profile in FCHL showed that the genotype is associated with serum MDA-LDL level, and the ratio of MDA-LDL and LDL-C, as well as TG, and the ratio of LDL-C and apo B. Together with the relationship between the genotype and HL activity in the subjects, these results on the lipid profile suggest that the common promoter polymorphism, C-514T, on the *HL* gene contributes to determine the serum MDA-LDL levels at least in male. The genetic contribution on the athrogenic phenotype is possibly caused by the decreased HL activities in the subjects. Thus, the polymorphism might be one of genetic factors modifying the progression of atherosclerosis via catabolism of TG-rich lipoproteins in FCHL.

The presence of a C-514T substitution with respect to the transcription start site of the *HL* gene accounts for 20–30% of the variance in the HL activity [14,19,20]. The frequency of the C allele is rather dominant in these above analyzed areas, and therefore, the clinical phenotype in homozygous subjects with T allele has not been fully clarified compared to those with C allele. Some studies have shown that the TG levels are similar among subjects with the three genotypes [14,18], and others have shown the increased (or increased tendency of) levels of plasma TG in the subjects with T/T genotype, compared to other two genotypes [16,17,28,29]. Recent study by Jansen, et al. showed that the genotype is associated with the increased pre-prandial and post-prandial plasma levels of particles containing both apo C-III and apo B (LpC-III:B) (17). Our results showed the significant increase in the serum TG levels in the patients with T allele compared to those with C allele clearly in male. The difference of effects on plasma TG level among studies is possible to be caused by the factors affecting the HL activity, such as gender, age, insulin sensitivity and obesity. Carr, et al. has recently shown that intraabdominal fat and sex steroid hormones contribute to the gender differences in HL activity [30]. Further studies on the lipoprotein metabolism in association with such affecting factors in the FCHL subjects are in progress.

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